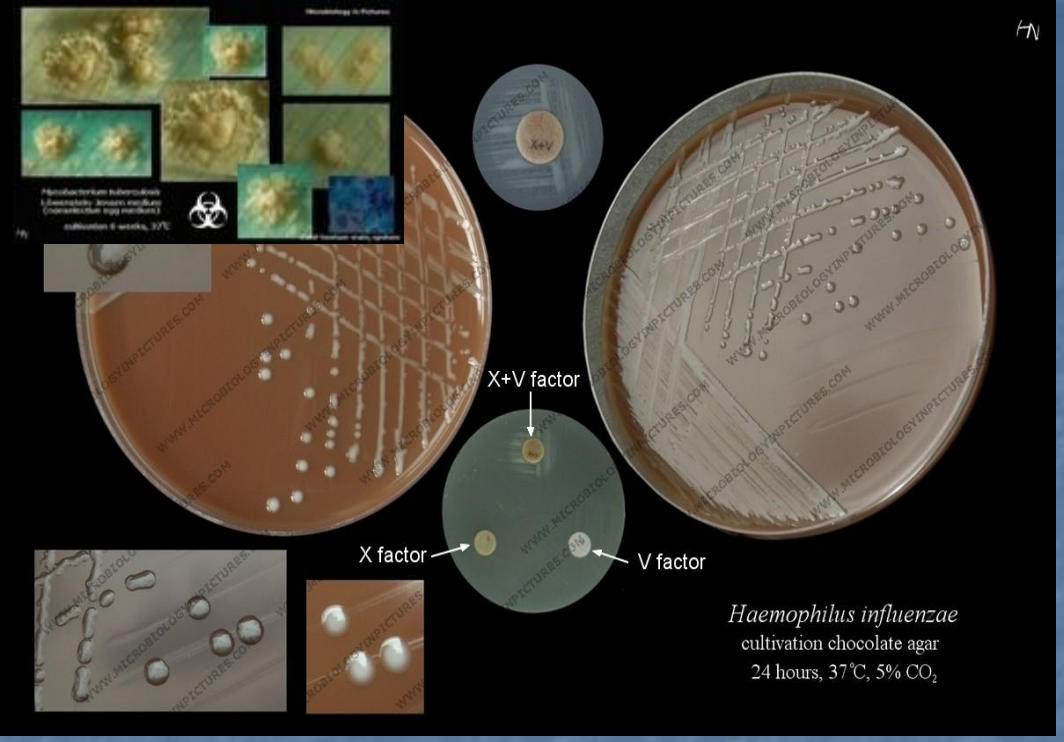


Haemophilus

Mycobactreium

General characteristics:

Genus Heamophilus

- Non intestinal Gram negative coccobacilli
- Fastidious bacteria (mostly)
- Grown under aerobic conditions or under slight CO2
- Non motile
- Non spore forming
- Usually capsulated
- Oxidase and catalase positive
- Facultative anaerobic

pathogenic as well as part of upper respiratory tract flora

*Heamophilus influenzae (*pneumonia, otitis media, epiglottitis, meningitis, bacteremia..)

- *Heamophilus aegypticus (*conjunctivitis)
- *Heamophilus ducreyi*
- *Heamophilus parainfluenzae*

Physiology and structure

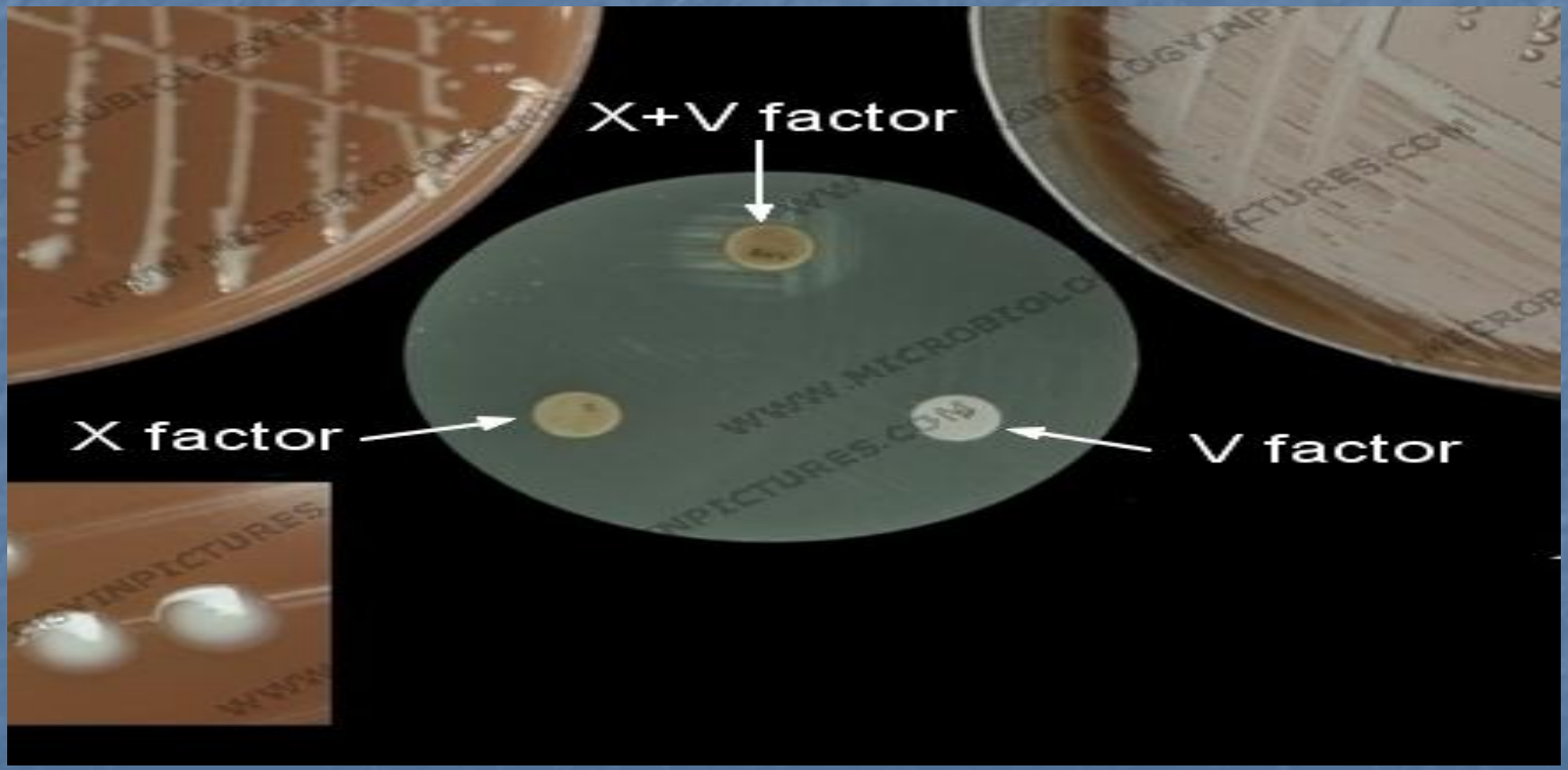
Growth-stimulating factors:

- Hemin (X factor)
- Nicotinamide adenine dinucleotide (NAD) (V factor)

Chocolate agar
Blood agar??

Heamophilus influenzae is the most important pathogen and have been subdivided according to :

1. Serotypes according to capsular antigens (a through f, the most important type b)


X+V factor
X factor
V factor

Pathogenesis and Immunity

- Capsule contains polyribitol phosphate (resist phagocytosis
- Lipopolysaccharide, low molecular weight glycopeptide in cell wall (impair ciliary function)
- Lipopolysaccharide lipid A (meningeal inflammation)
- IgA1 proteases produced by bacteria

Diseases

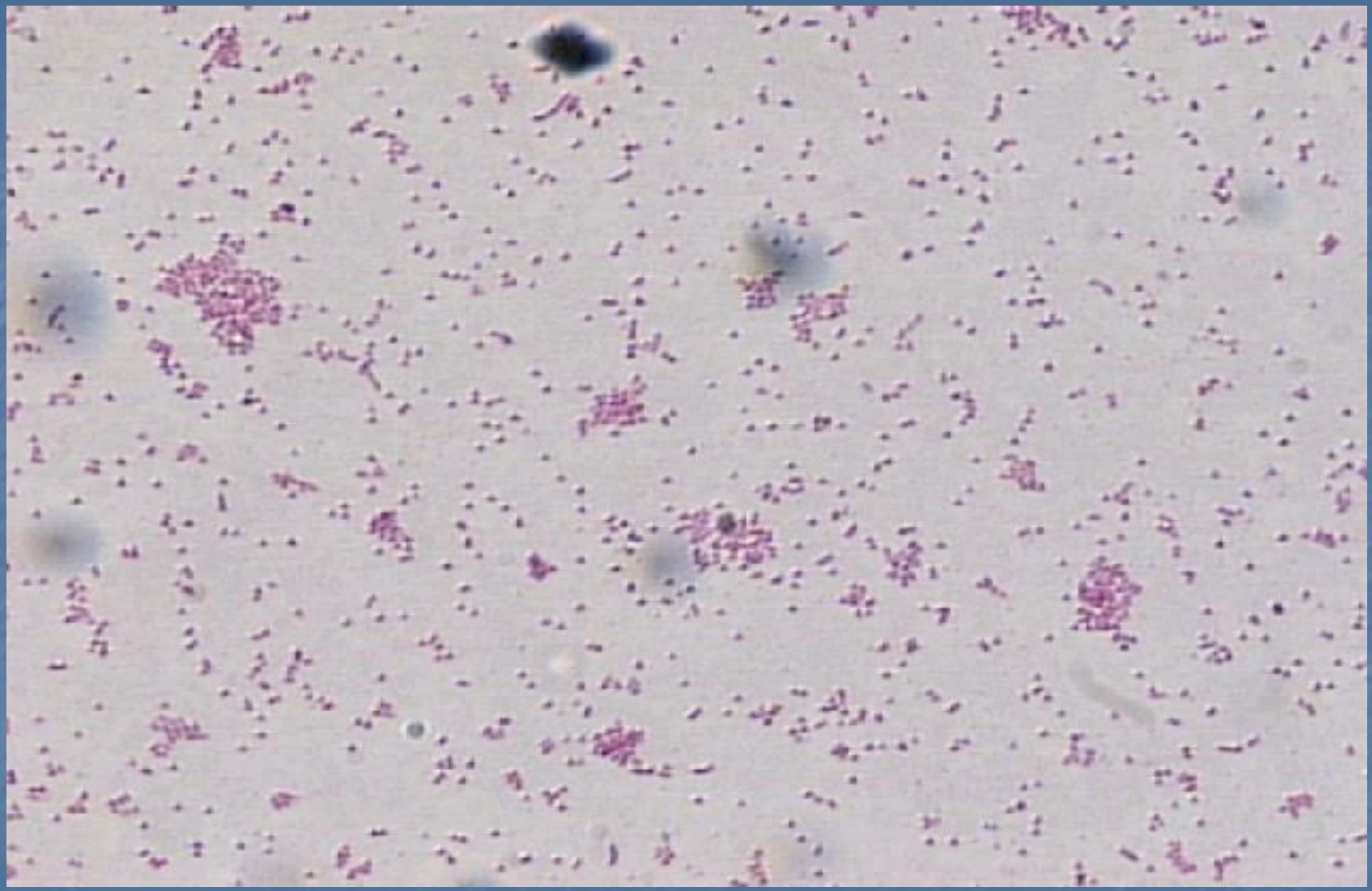
- Meningitis
- Pneumonia
- Otitis media
- Conjunctivitis (*H. aegypticus*)
- and other infections

Lab diagnosis

- Specimen
- Microscopy
- Culture

Diagnosis of *Haemophilus*

- **Specimen:**
  - according to site of infection; swap, sputum, CSF,
- **Stain:** Gram negative coccobacilli



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- **Culture:**
- *H. influenzae* grow on blood agar or chocolate agar as it requires X factor and V factor that found on blood
- ***H. parainfluenzae* requires only V factor.**
- **On Blood agar:** A 24 h colony of *H. influenzae* on blood agar is very small usually non hemolytic
- **On chocolate agar:** A 24 h colony of *H. influenzae* on chocolate agar is larger than that observed on blood agar

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Haemophilus influenzae and Staphylococcus aureus. Satellite growth on blood agar

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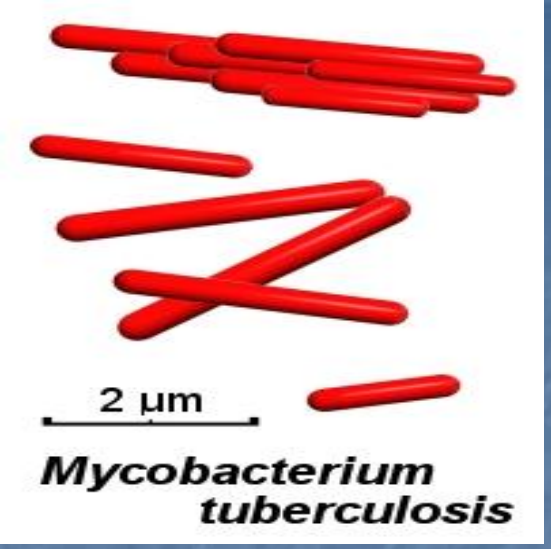
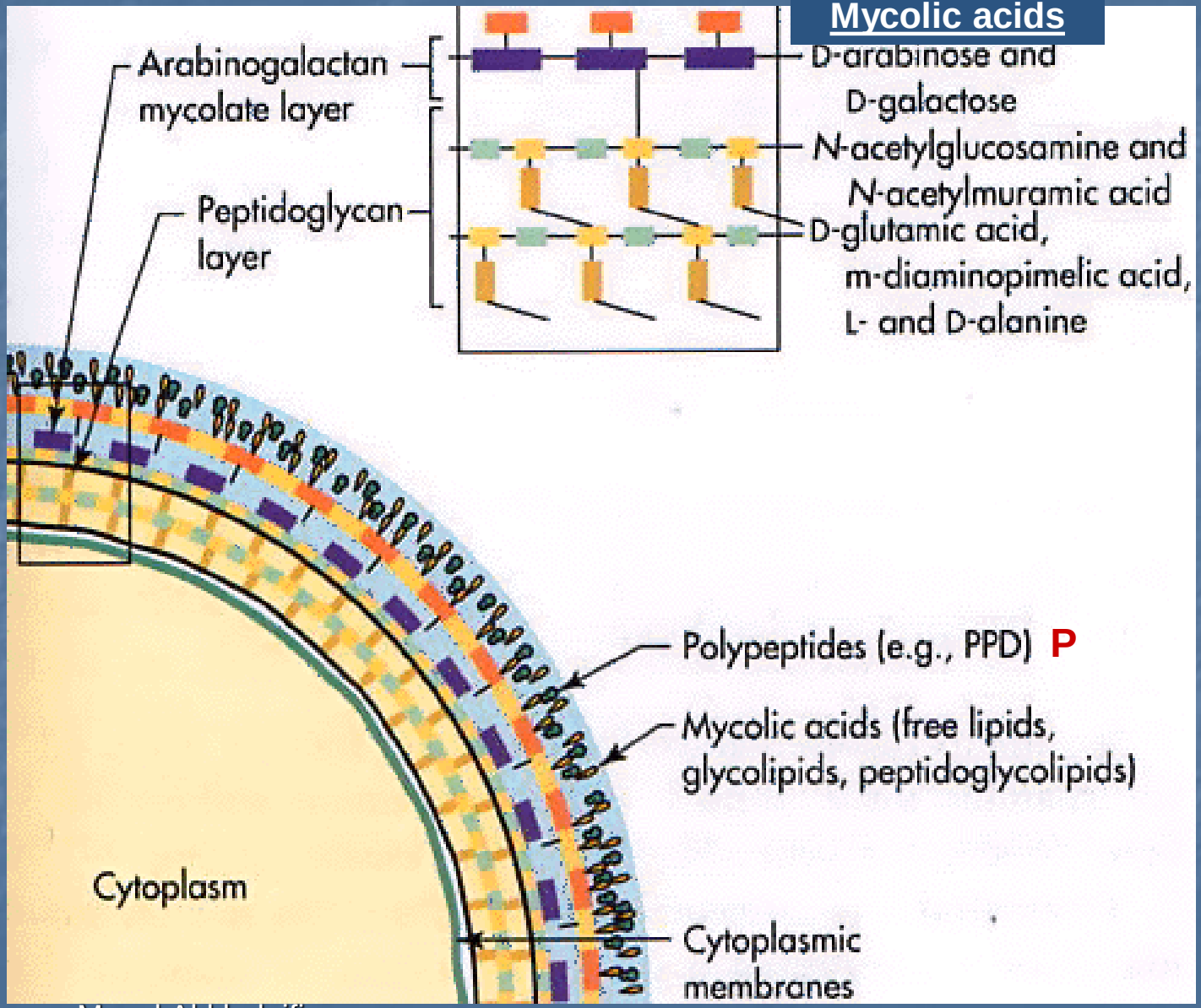


Acid-fast bacteria
Mycobacterium

- include the *Mycobacterium* and few species of *Nocardia*.
- The Mycobacterium include 2 species:
  - *Mycobacterium tuberculosis*, which causes tuberculosis
  - *Mycobacterium leprae,* which causes leprosy (uncommon)
- *Mycobacterium* contains 40% lipid content in their cell envelop.
- High lipid content→ difficult to stain by ordinary dye but requires special dye as Carbol fuchsin and heating, and once stained are difficult to decolorize with acid-alcohol mixture.
- Acid fastness is due to high lipid content of cell envelop

Lipid-Rich Cell Wall of Mycobacterium



Unusual cell wall lipids (mycolic acids,etc.)

Protein **D**erivative)

- **Morphology, metabolism and characters:**
  - Pleomorphic rods, slender (thin) straight or slightly curved rods
  - Acid fast stain
  - Mycobacteria are Gram-positive
  - Non-motile
  - Non-spore forming
  - Non-capsulated
  - Obligate aerobic
  - Catalase positive
  - Most *Mycobacteria* are found in habitats such as water or soil. However, a few are **intracellular pathogens** of animals and humans.

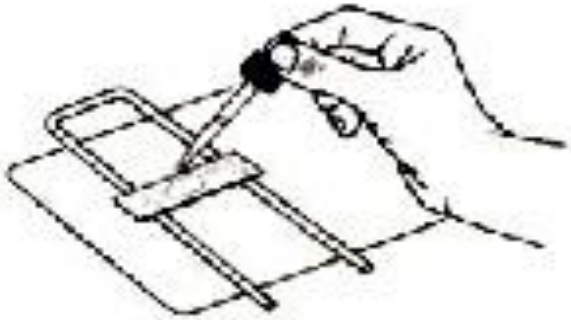
Lab Diagnosis

- Specimen
- Acid fast stain
- cultures

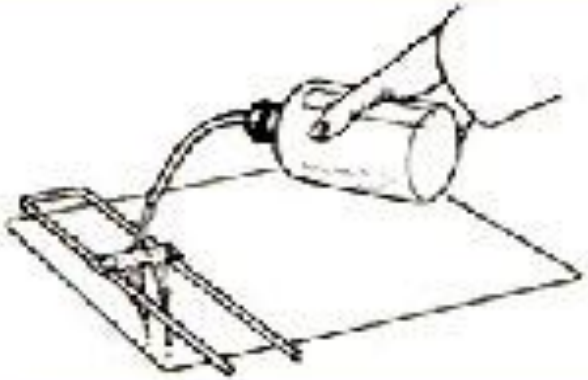
1 Cover smear with carbolfuchsin.Steam over boiling water for 8 minutes. Add additional stain if stain boils off.

2 After slide has cooled decolorize with acid-alcohol for 15 to 20 seconds.

3 Stop decolorization action of acid-rinsing briefly with water.



4 Counterstain with methylene blue for 30 seconds.



5 Rinse briefly with water to remove excess methylene blue.

6 Blot dry with bibulous paper. Examine directly under oil immersion.

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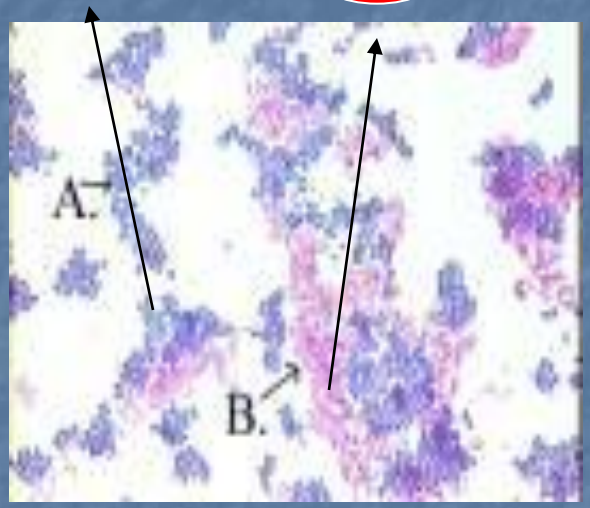
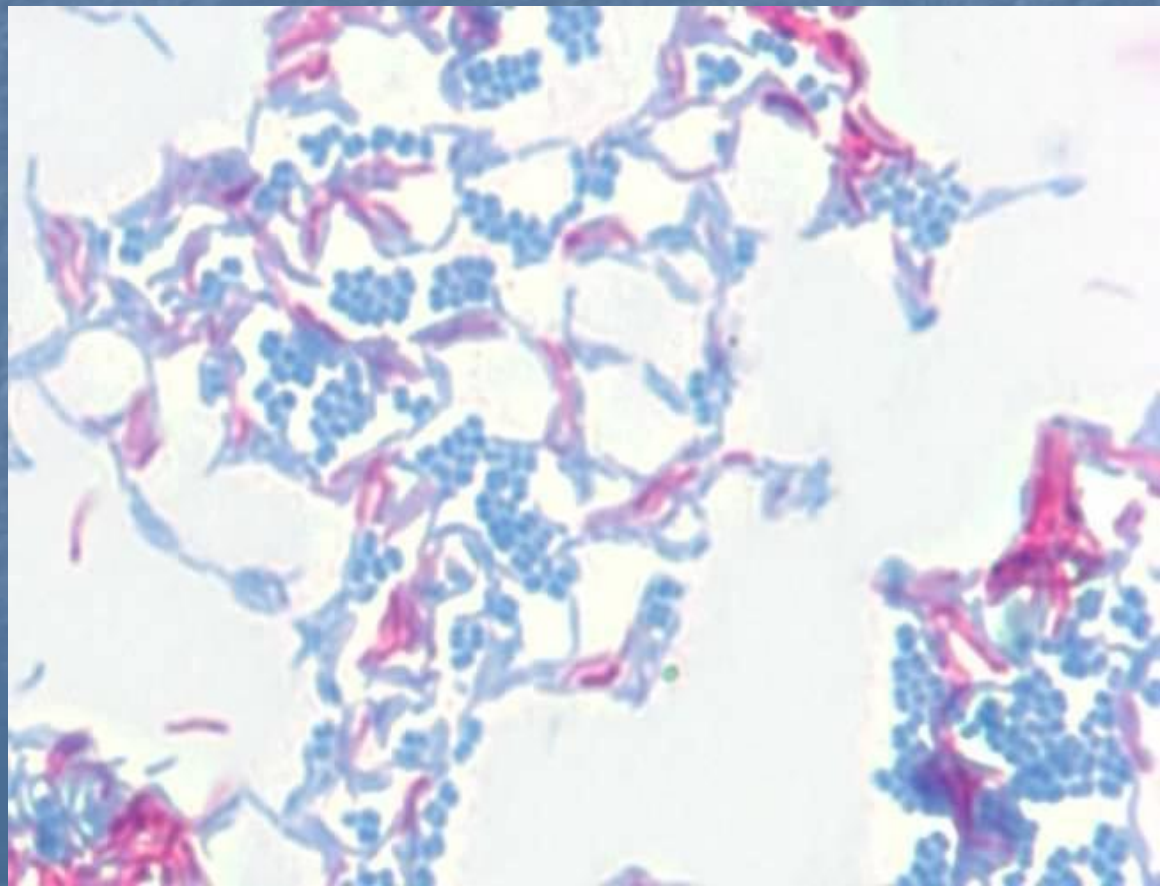
Ziehl-Neelsen acid-fast staining procedure

Example of Acid-Fast bacteria

Blue=Non acid-fast bacteria

Red= acid fast bacteria

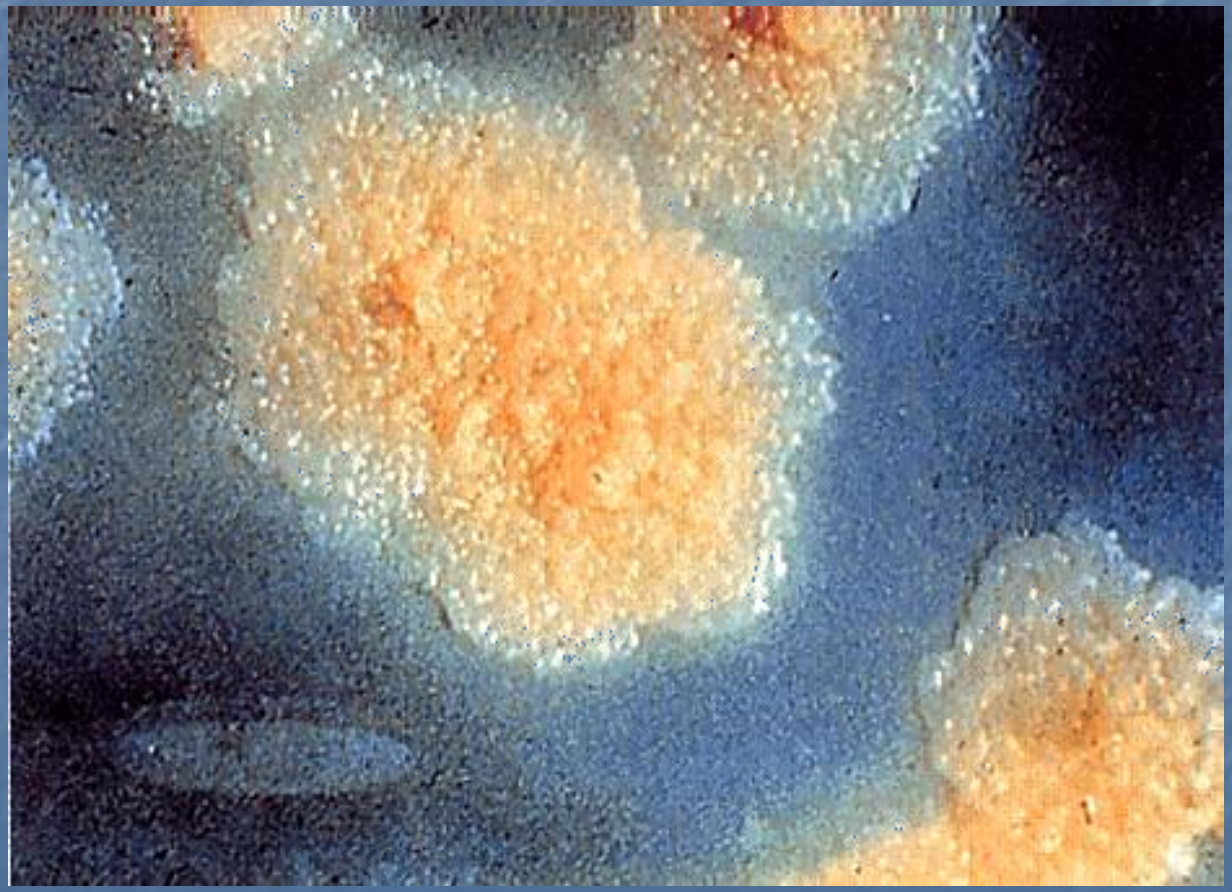




Acid fast organisms stain red or pink

Non acid fast organisms and tissue cells stain blue.

Eight Week Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Agar



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