

Practical 6

Haemophilus

- The term *Haemophilus* represents a large group of Gram-negative rods that like to grow on chocolate blood agar and restricted to organisms that are dependent on one or both of certain growth promoting substances termed X (haematin) and V (NAD) which present in blood; therefore these organisms grow only on blood agar or chocolate agar.

➤ **General characters:**

1. Non intestinal pleomorphic Gram negative coccobacilli
2. Grown under aerobic conditions or under slight CO₂ tension (5% CO₂)
3. Non motile
4. Non spore forming
5. Usually capsulated (polyribitol phosphate)
6. Oxidase and catalase positive
7. Fastidious bacteria (mostly)
8. Facultative anaerobic

➤ **Species of *Haemophilus*:**

- *H. influenzae*
- *H. parainfluenzae*
- *H. aegypticus*
- *H. ducreyi*

➤ ***H. influenzae*:**

- *Haemophilus influenzae* is the most important pathogen and have been subdivided according to :
Serotypes according to capsular antigens (a through f, the most important type b)

- **Non capsulated strains** colonize the nasopharynx in up to 80% of healthy individuals in the upper respiratory tract and become a secondary pathogen on respiratory mucosa that has become susceptible to bacterial attack after primary influenzae virus infection.
 - Non capsulated strains are less invasive, but they are apparently able to induce an inflammatory response that causes disease.
 - It may spread locally and cause pneumonia, otitis media, and sinusitis
- **Encapsulated strains**, are a very minor colonized in 5% of healthy individuals in the upper respiratory tract.
 - Encapsulated organisms can penetrate the epithelium of the nasopharynx and invade the blood capillaries directly.
 - Their capsule allows them to resist phagocytosis

- It cause pediatric meningitis, otitis media & epiglottitis (obstructive laryngitis)
- *H. influenzae* requires both X and V factors

➤ **H. parainfluenzae:**

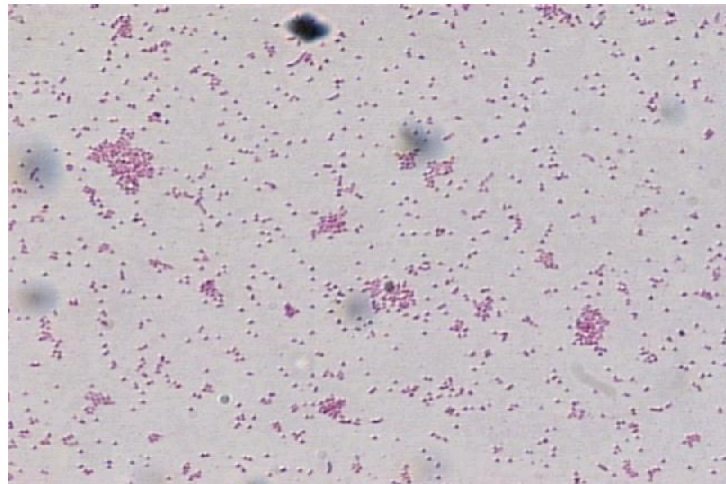
- Similar to non capsulated strain of *H. influenzae*
- This organism needs only V factor

➤ **Diagnosis of Haemophilus:**

○ **Specimen:**

according to site of infection; swap, sputum, CSF, discharge

Stain: Gram negative coccobacilli



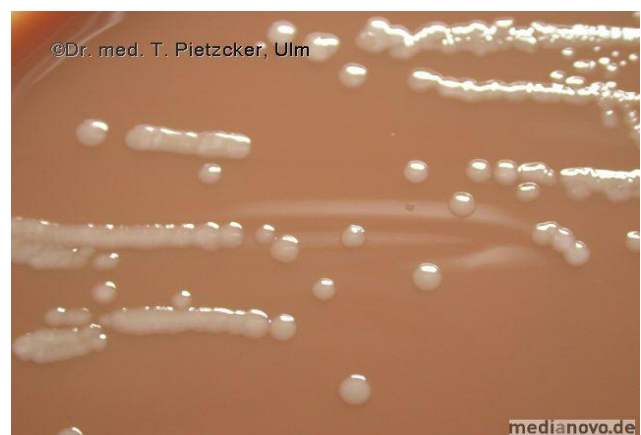
Culture:

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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X and F factors

Satellitism:

Blood agar contains much X factor and little V factor and so the presence of another microorganism such as *Staph. aureus* which produces V factor will support the growth of *H. influenzae* i.e. larger colonies of *H. influenzae* are observed near *Staph. aureus* colonies.

Capsule swelling: Specific antiserum added to a slide of the organism allows swelling of the bacterial capsule thus permitting rapid diagnosis of *H. influenzae* in sputum.

Haemophilus influenzae and *Staphylococcus aureus*. Satellite growth on blood agar



Acid-fast bacteria

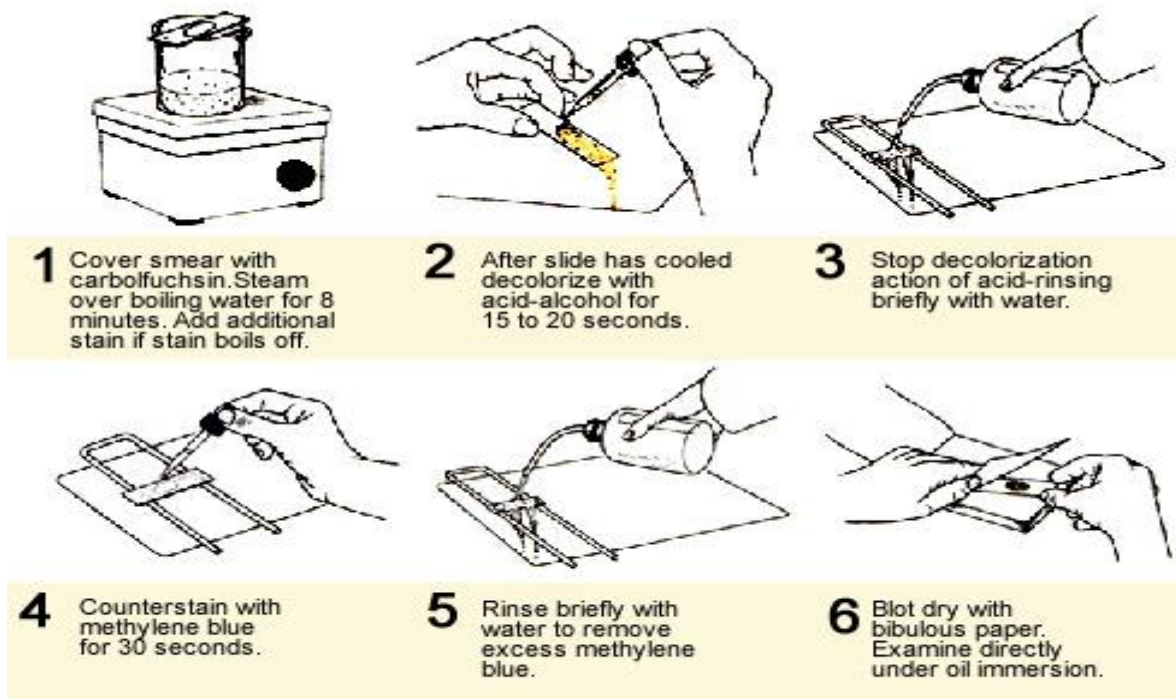
- Acid fast bacteria include the *Mycobacterium* and few species of *Nocardia*.
- The *Mycobacterium* include 2 species:
 - *Mycobacterium tuberculosis*, which causes tuberculosis
 - *Mycobacterium leprae*, which causes leprosy
- *Mycobacterium* contains 40% lipid content in their cell envelop.
- High 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

Morphology, metabolism and characters:

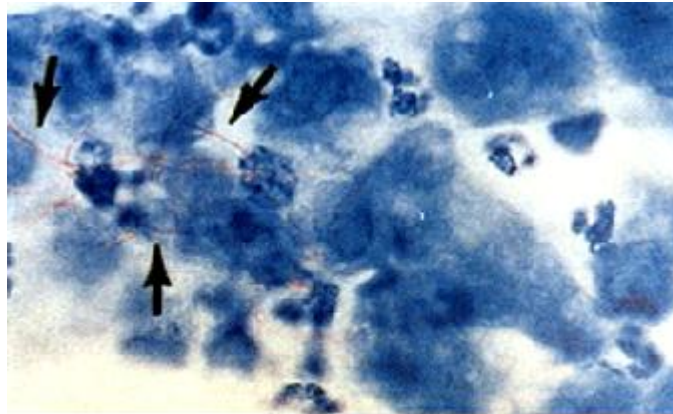
- Pleomorphic rods, slender (thin) straight or slightly curved rods, 2-4 μ long x 0.2-0.5 μ in diameter
- Acid-alcohol fast (Acid fast stain)
- Mycobacteria are [Gram-positive](#)
- [Non-motile](#)
- Non-spore forming
- Non-capsulated
- Obligate aerobic
- Catalase positive
- Most *Mycobacteria* are found in habitats such as water or soil. However, a few are **intracellular pathogens** of animals and humans.

Ziehl Neelsen Acid fast Stain method

Acid fast organisms contains waxlike lipoid material affecting staining quality. Carbol fuchsin is primary stain. Acid fast organisms resist decolorization with acid alcohol. After decolorization, methylene blue is added to organisms to counterstain any material that is not acid fast.



Ziehl-Neelsen acid-fast staining procedure



Mycobacterium
Acid Fast Stain

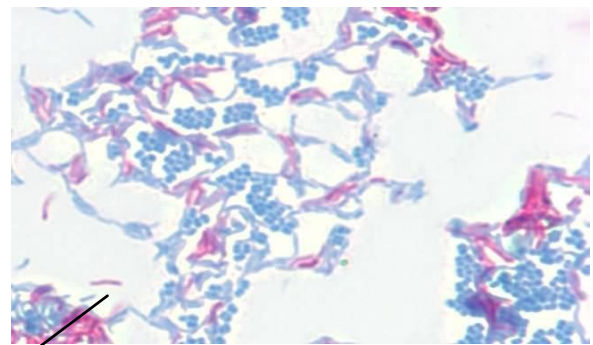
Acid fast organisms
stain red or pink

Non acid fast
organisms and tissue
cells stain blue.



Acid fast stain

(mycobacterium tuberculosis)



Lab diagnosis:

- TB is obligate aerobes → infect lung → rich in oxygen
- They can grow on simple liquid medium
- They grow on complex enriched & selective media e.g.
- **Direct methods:**
 - **Microscopical examination:** → Acid-fast bacteria
 - **Cultures :**
 - Bactec radiometric culture: a liquid broth in bottle , with radioactive palmitate as a carbon source
 - Mycobacteria grow and use the carbon, allowing early detection (1-2 weeks) even before colonies can be seen
 - **Lowenstein-Jensen medium** which is made by addition of malachite green & some antibiotics which inhibit the growth of fungi and other bacteria.
 - Slow growing organism → generation time each 20 h → need 4 weeks for growth.
 - Presence of CO₂ enhances the growth.
 - Optimum growth temp is 37°C.



Eight Week Growth of *Mycobacterium tuberculosis* on Lowenstein-Jensen Agar

- **Indirect methods:** used
 - When direct method give negative result **or**
 - When there is no available material for direct examination
- **PCR and DNA probes**
- **Chest X-ray**
- **Pathogenesis of TB:**
 - **I- Primary tuberculosis:**
 - The first exposure to *M. tuberculosis* is called primary tuberculosis
 - TB is a highly communicable disease that is transmitted mainly by droplets due to *M. tuberculosis* causing **pulmonary infection** or by ingestion of cow milk infected with *M. bovis* resulting in **intestinal infection**
 - Primary tuberculosis may occur at any epithelial site but common in lung
 - **II- Secondary or Reactivation infection:**
 - This is usually caused by endogenous TB that have survived in the primary lesion in less than 5% of those infected and rarely due to exogenous infection
 - Reactivation is seen primarily in immuno-compromised patients
 - It is characterized by chronic tissue lesions, formation of tubercles, caseation and fibrosis
 - Reactivation lesions occur at apex of lung and other well oxygenated organs such as brain, kidney
- **A. Clinical manifestations:**
 - Fever, fatigue, night sweats, and weight loss are common.
 - Cough and bloody sputum are common in pulmonary TB

Tuberculin test (PPD test):

- This is a skin test which is useful in diagnosis of TB specially during the primary infection where the majority of cases are symptom less
- During primary infection the host acquires hypersensitivity to TB and becomes positive to tuberculin
- It is carried out by intradermal injection of 0.1ml of purified protein derivative (tuberculin) in the forearm
- Red indurations are observed in positive test, measuring not less than 10 mm in diameter appears after 2-3 days after injection. The best time for reading is 72 hours after injection.