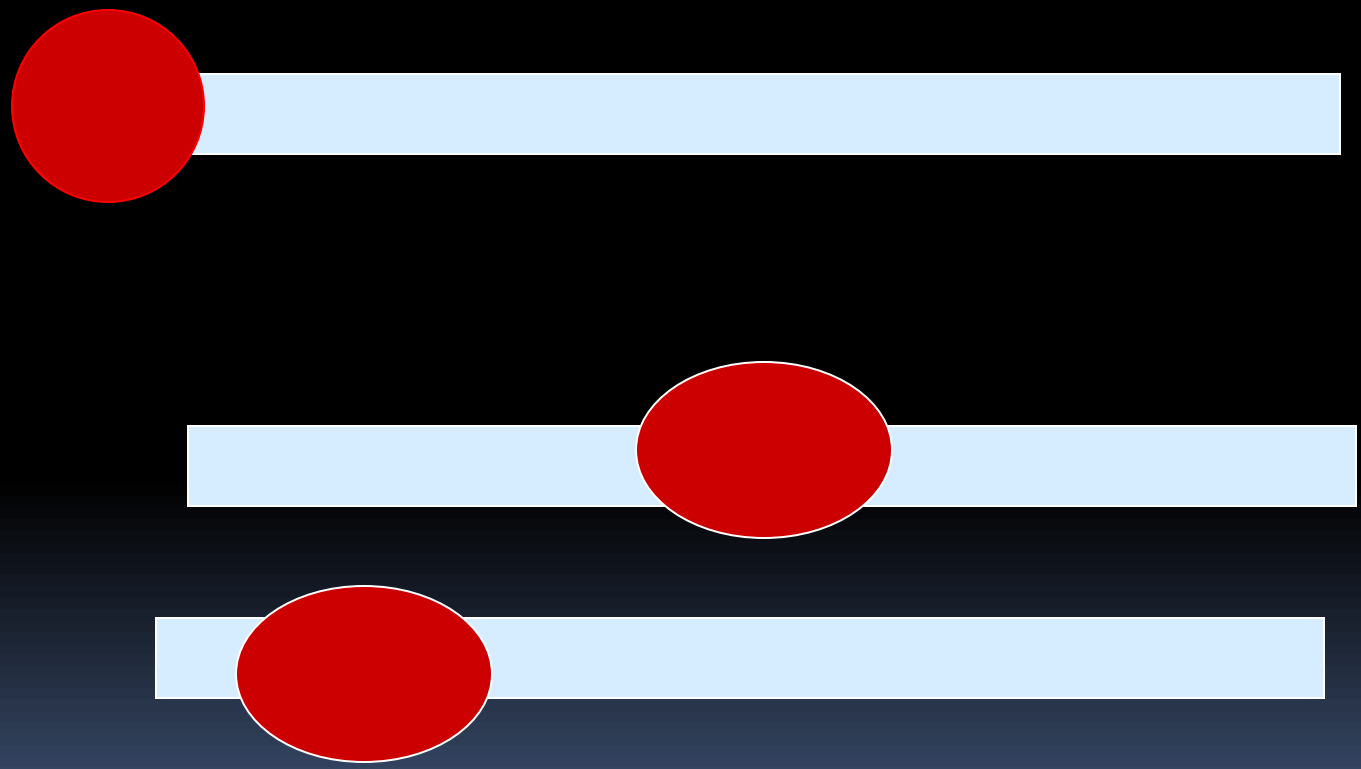
Clostridia

- Large Gram positive
- Straight or slightly curved rods with slightly rounded ends
- Anaerobic bacilli
- Spore forming
- Saprophytes
- Some are commensals of the animal & human gut which invade the blood and tissue when host die and initiate the decomposition of the corpse (dead body)
- Causes diseases such as gas gangrene, tetanus, botulism & pseudo-membranous colitis by producing toxins which attack the neurons pathways



Most are obligate anaerobes produce neuro histo toxins

Clostridia of medical importance

Clostridium
Causing

Tetanus
e.g. *Cl. tetani*

Gas gangrene

Botulism
e.g. *Cl. botulinum*

Antibiotic associated diarrhea
e.g. *Cl. difficille*

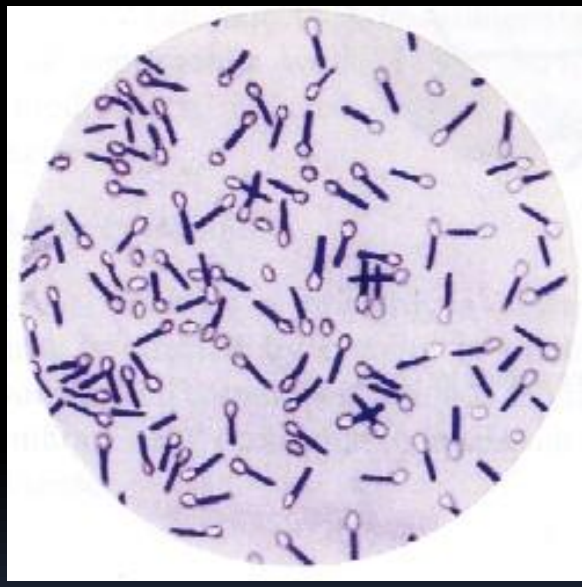
Saccharolytic
e.g. *Cl. perfringens* & *Cl. septicum*

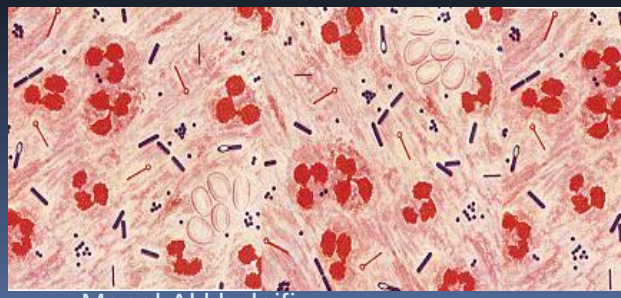
Proteolytic
e.g. *Cl. sporogenes*

Mixed: *Cl. histolyticum*

Clostridium Causing Tetanus *Cl. tetani*

- Gram positive
- Form round, terminal endospore (drumstick with a large round end)
- Fermentative
- Obligate anaerobe
- Motile by peritrichous flagella
- Grows well in cooked meat broth and enriched blood agar
- Spores are highly resistant to adverse conditions
- Iodine (1%) in water is able to kill the spores within a few hours



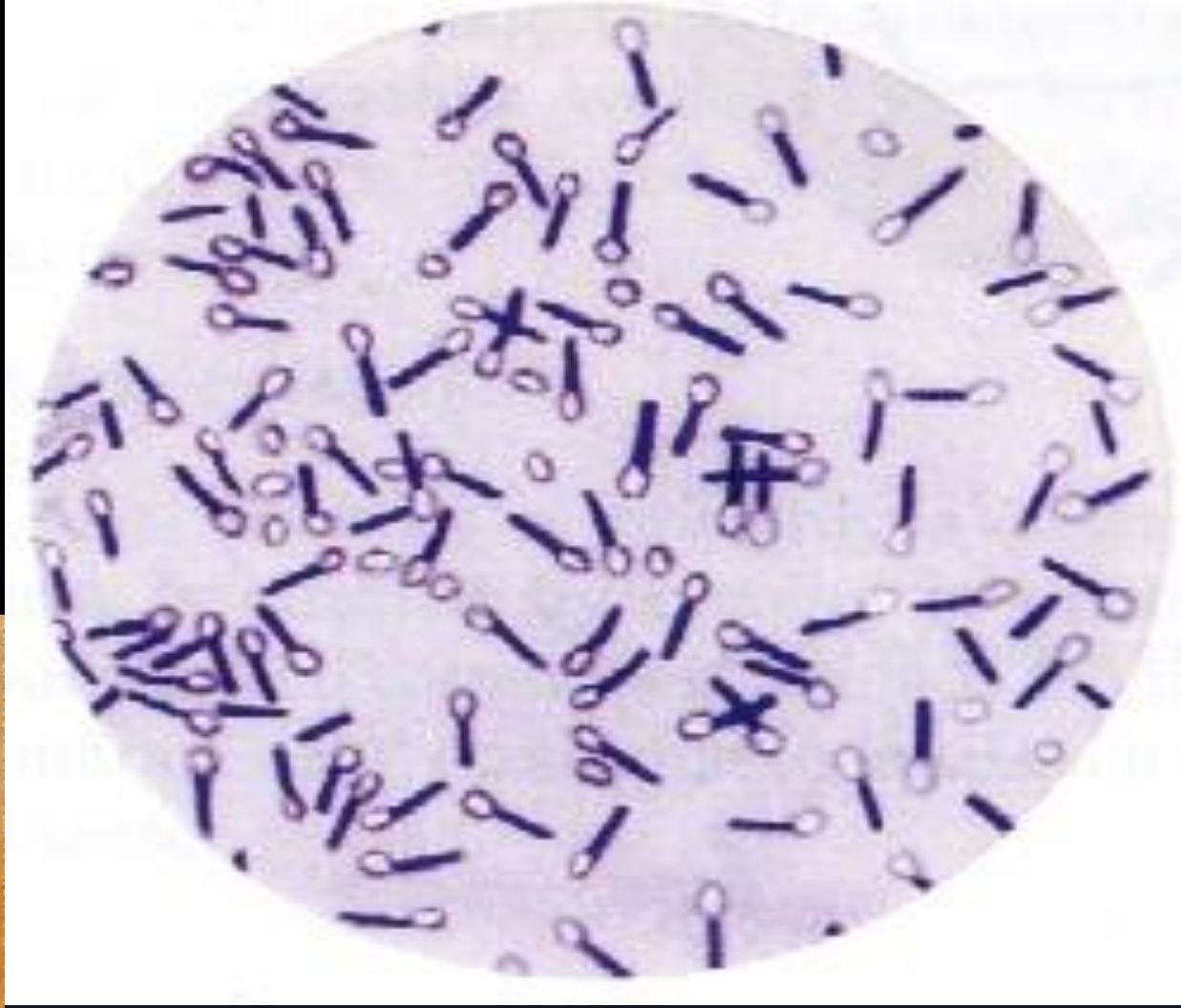


Toxins

- *Cl. tetani* produces two types of toxins:
  - **Tetanolysin**, which causes lysis of RBCs
  - **Tetanospasmin** is neurotoxin and essential pathogenic product

Laboratory Diagnosis of Tetanus

- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.
- **Specimen:** Wound exudates using capillary tube
- **Culture:**
  - On blood agar and incubated anaerobically
- Gram stain is a good method for identifying *Clostridium*
  - *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance



Clostridium Causing Gas Gangrene

Clostridia causing gas gangrene

Saccharolytic organisms
Cl. perfringens, Cl. septicum
Ferment carbohydrates
Acid and gas are produced

Proteolytic organisms
Cl. sporogenes
Digest proteins with blackening bad smell production

Mixed saccharolytic & proteolytic
Cl. histolyticum

Saccharolytic Microorganisms

Cl. perfringens
Causing

Gas gangrene

Food poisoning
(Enterotoxin)

Clostridium perfringens

- Large Gram-positive bacilli with stubby ends

(Does not sporulate on ordinary media)

- **Capsulated**
- **Non motile (*Cl. tetani* is motile)**
- Anaerobic
- Grown quickly on selective media
- **Can be identified by** Nagler reaction

Toxins

- **The toxins of *Cl. perfringens***
  - **α toxin** is the most important toxin
    - Lyses of RBCs, platelets, leucocytes and endothelial cells
  - **β-toxin** is responsible for necrotic lesions
  - **Enterotoxin** is heat labile toxin produced in colon → food poisoning

Laboratory Diagnosis

- **Specimen:** Histological specimen or wound exudates
  - Specimens of exudates should be taken from the deeper areas of the wound where the infection seems to be most pronounced
- **Microscopical examination (Gram, Spore stain)**
  - Gram-positive bacilli, **non motile**, **capsulated** & sporulated
  - The spore is oval, sub-terminal & non bulging
  - Spores are rarely observed
- **Culture:** Anaerobically at 37C
  - **On cooked meat medium** → blackening of meat will observed with the production of H_2S and NH_3
  - **On blood agar** → β-hemolytic colonies

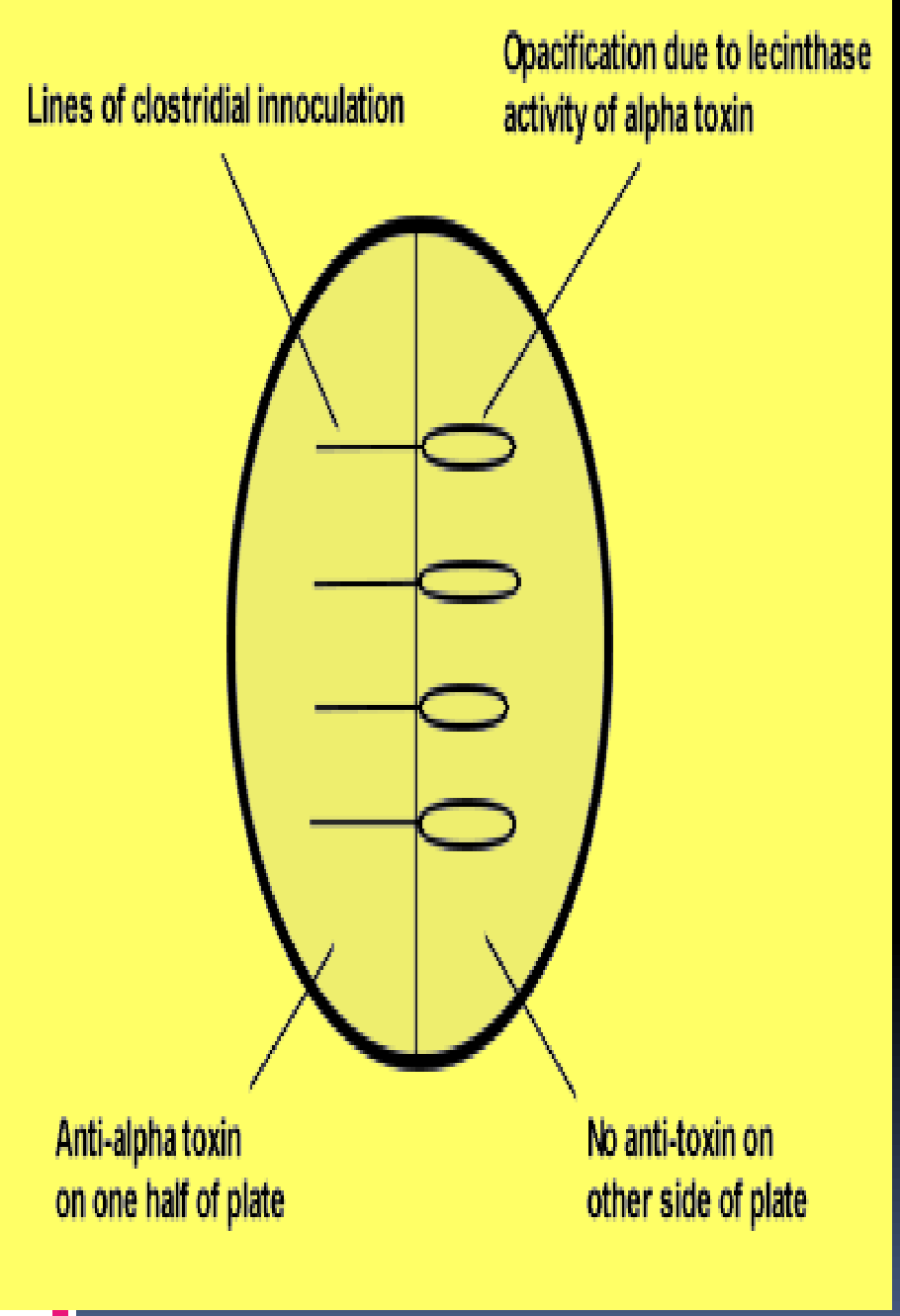
Biochemical Tests

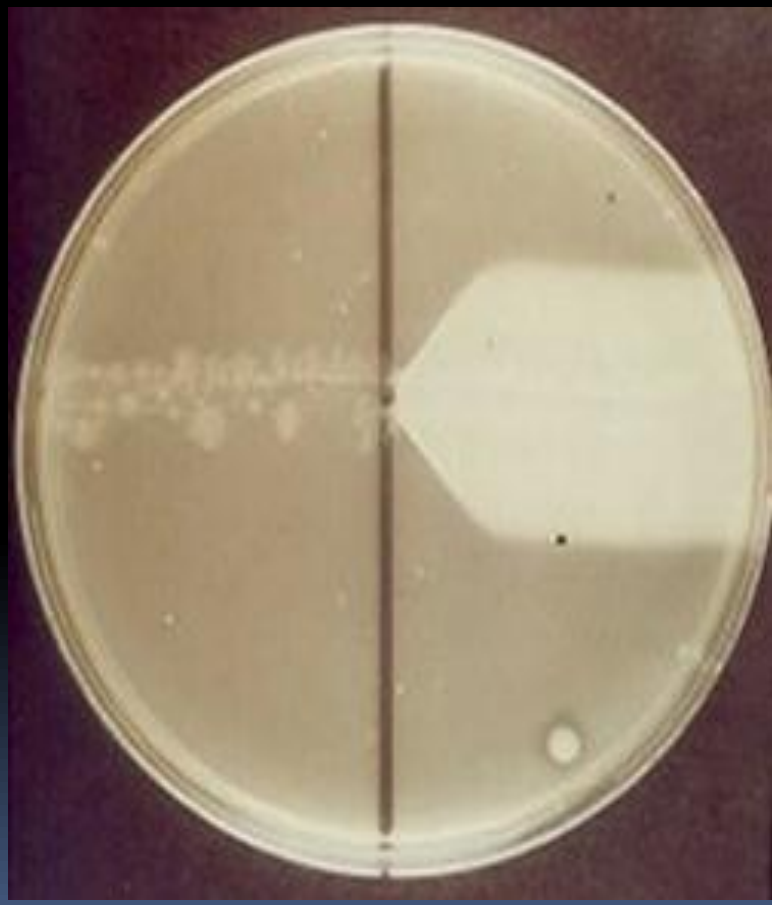
- *Cl. perfringnes* characterized by:
  - It ferments many carbohydrates with acid & gas
  - It acidified litmus milk with stormy clot production
  - Nagler reaction is positive

Nagler's Reaction

- This test is done to detect the lecithinase activity
  - inoculated on the medium containing human serum or egg yolk (contains lecithin)
  - The plate is incubated anaerobically at 37 C for 24 h
  - Colonies of *Cl. perfringens* are surrounded by zones of turbidity due to lecithinase activity and the effect is specifically inhibited if *Cl. perfringens* antiserum containing α antitoxin is present on the medium

Nagler Reaction





Procedure of Nagler Reaction

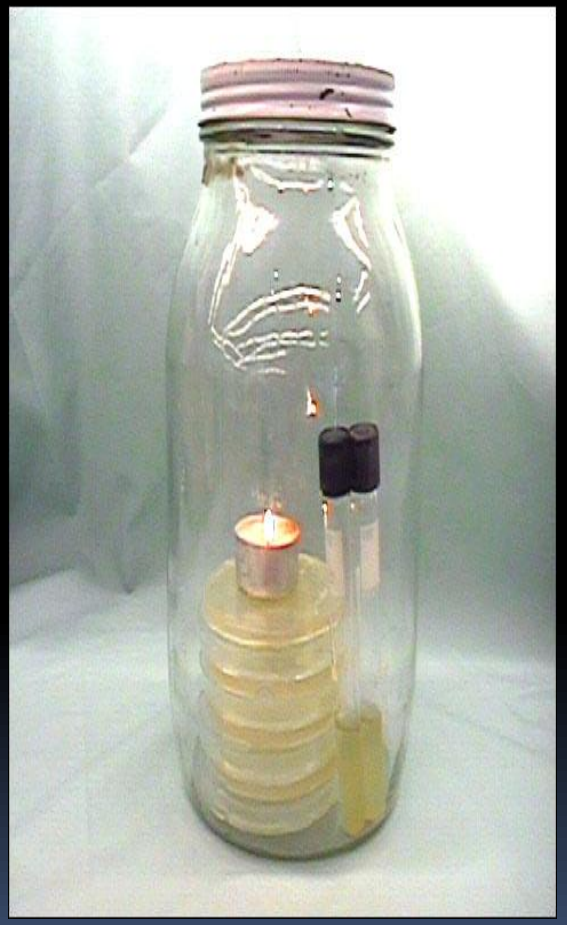
Positive Nagler Reaction

Anaerobic Cultivation

- Removal of oxygen & replacing it with inert gas
  - Anaerobic Jar
    - It is especially plastic jar with a tightly fitted lid
    - Hydrogen is introduced from commercially available hydrogen generators envelop
    - 10 ml of water is added to envelop immediately before placing it in the jar
    - Hydrogen and carbon dioxide will release and react with oxygen in the presence of catalyst to form water droplet
    - Anaerobic indicator (Methylene blue) is placed in the jar
    - Methylene blue is blue in oxidized state (Aerobic condition) while turns colorless in reduced state (Anaerobic condition)

Anaerobic Jar

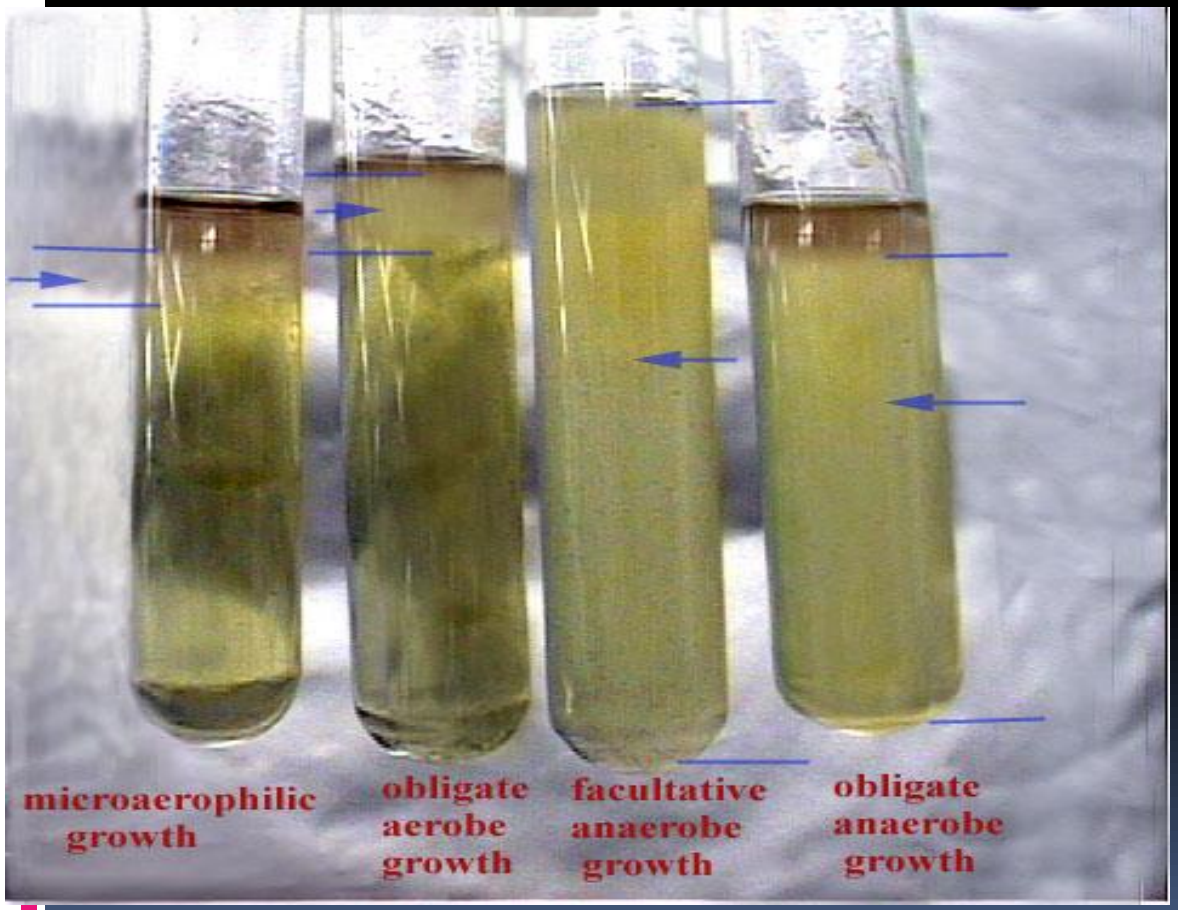
Candle Jar



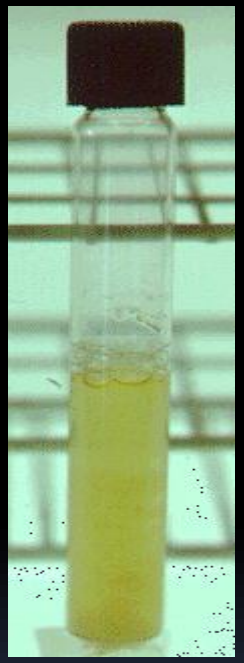
Anaerobic Cultivation

- Culture Media containing reducing agent
  - **Thioglycollate broth**
    - It contains
      - Sodium thioglycollate (Reducing agent)
      - Rezazurin (redox indicator)
      - Low percentage of Agar-Agar to increase viscosity of medium
  - **Cooked Meat Medium**
    - It contains
      - Meat particles (prepared from heart muscles) which contain hematin & glutathione that act as reducing agent

Growth on Fluid Thioglycolate



Clostridium Growing in Thioglycolate Medium



Reducing agents in the medium absorb oxygen and allow obligate anaerobes to grow

Reaction on Cooked Meat Medium

- Saccharolytic reaction
  - It causes fermentation of glycogen of muscles
  - Production of acid and gas
  - Meat particles remain intact
  - e.g *Cl. perfergines*
- Proteolytic Reaction
  - It causes digestion of meat particles
  - Formation of black, foul smelling due to sulfur compounds