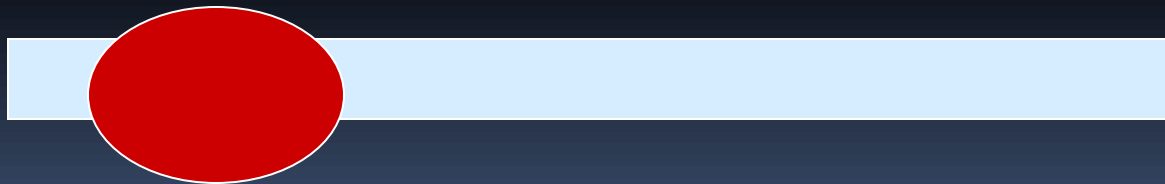
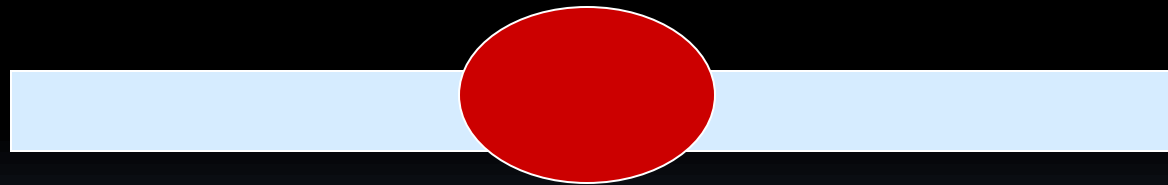


# Clostridia

- 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. difficile*

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

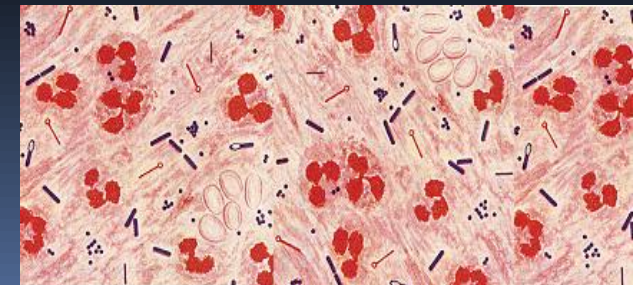
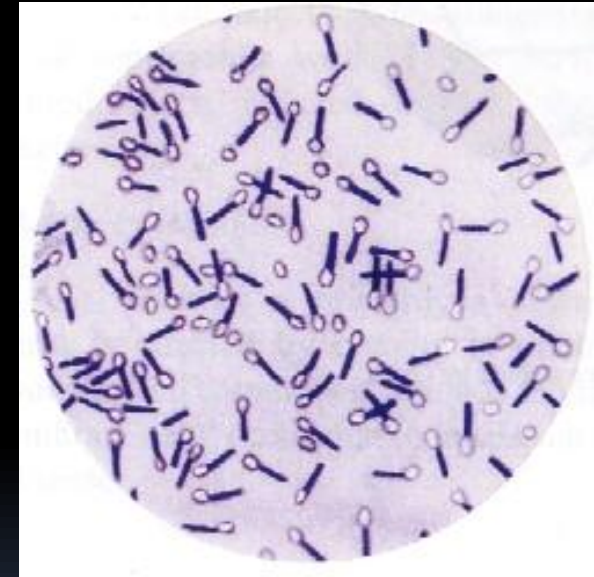
**Proteolytic**  
e.g. *Cl. sporogenes*

**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

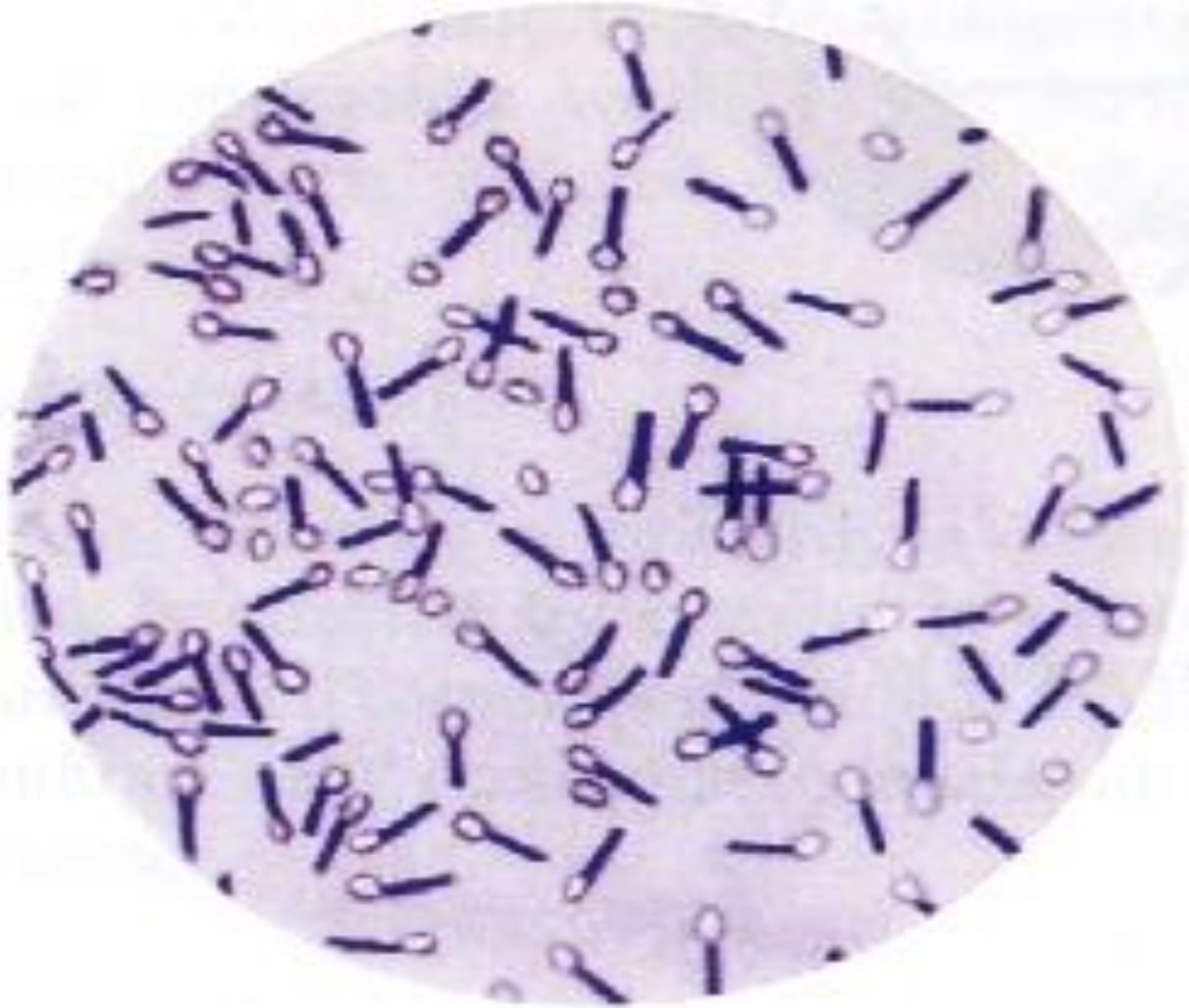


# Toxins

- *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*
  - $\alpha$  toxin is the most important toxin
    - Lyses of RBCs, platelets, leucocytes and endothelial cells
  - $\beta$ -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 be observed with the production of H<sub>2</sub>S and NH<sub>3</sub>
  - **On blood agar** → β-hemolytic colonies

# Biochemical Tests

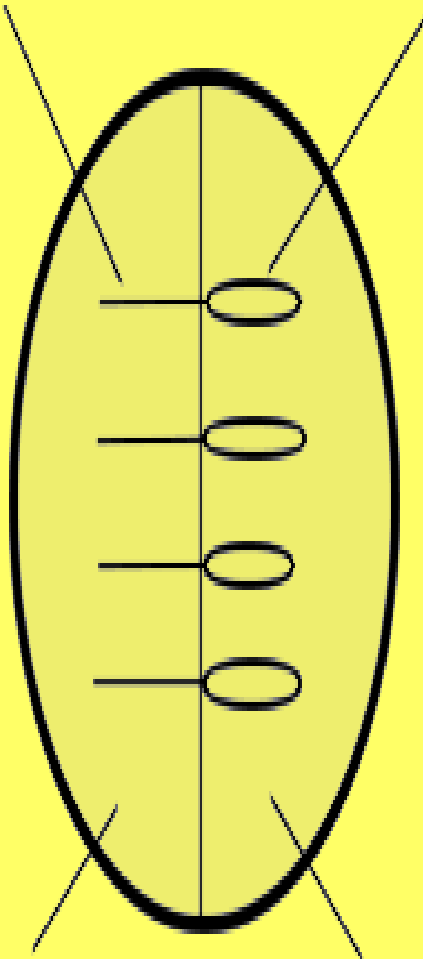
- *Cl. perfringens* characterized by:
  - It ferments many carbohydrates with acid & gas
  - It acidified litmus milk with stormy clot production
  - Nagler reaction is positive

# 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  $\alpha$  antitoxin is present on the medium

Lines of clostridial inoculation

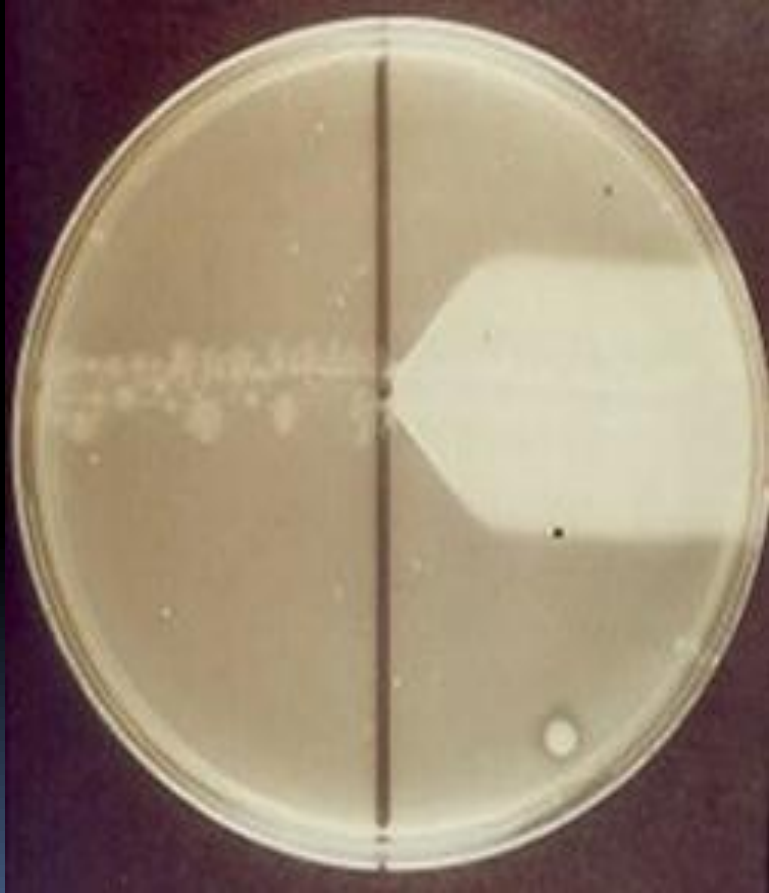
Opacification due to lecithinase activity of alpha toxin



Anti-alpha toxin  
on one half of plate

No anti-toxin on  
other side of plate

# Nagler Reaction



Procedure of Nagler Reaction

Positive Nagler Reaction

Maha Alkhulaini

# Anaerobic Cultivation

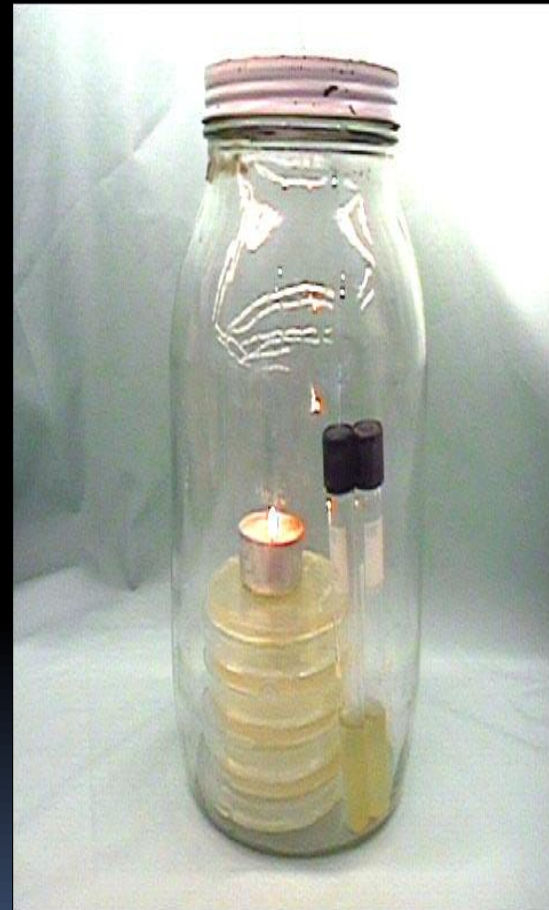
- Removal of oxygen & replacing it with inert gas
  - Anaerobic Jar
    - It is especially plastic jar with a tightly fitted lid
    - Hydrogen is introduced from commercially available hydrogen generators envelop
    - 10 ml of water is added to envelop immediately before placing it in the jar
    - 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

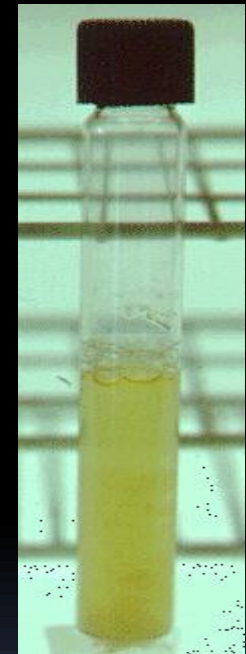
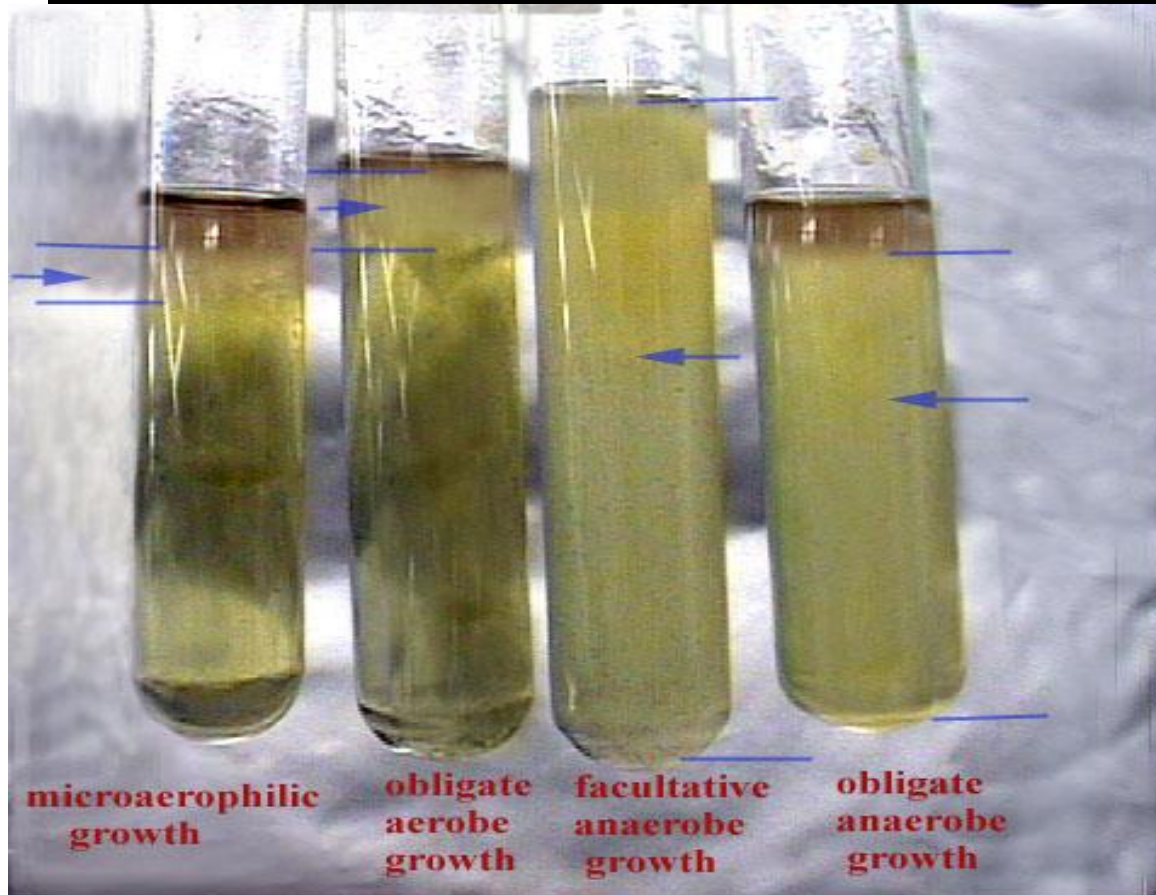


# Anaerobic Cultivation

- 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