Lab – 12

Genus: *Clostridium spp.*

Genus: *Clostridium*

G + ve Bacilli

Anaerobic spore forming

Genus: *Clostridium*

4 important species:

C. tetani

C. botulinum

C. perfringens

C. difficile

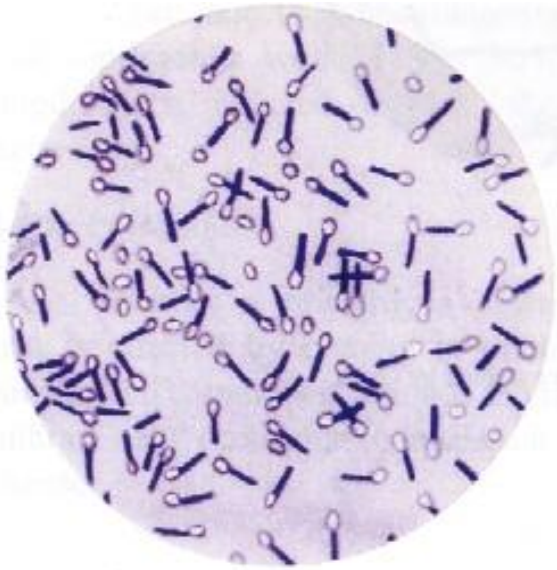
Clostridium is G+ve, obligate anaerobic spore-forming bacilli.

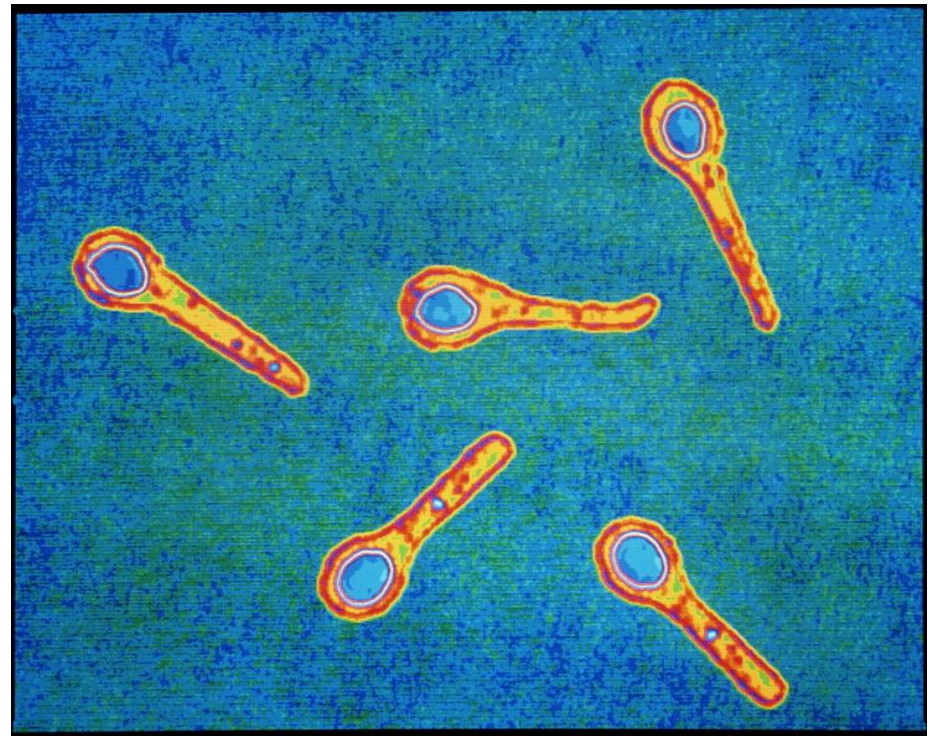
Four main species responsible for disease in humans:

I// Clostridium tetani

- Rod-shaped, rounded ends.
- Motile with peritrichous flagella
- **Spores are rounded terminal, form drum-stick appearance.**
- Spores are highly resistant, may remain alive for years, and resist boiling for 15-90 min., some may resist dry heat at 150^0C for 1 hour.

Clostridium tetani





C. tetani **their spores are terminally location**

II. Clostridium botulinum

- Rod-shaped, single or in pairs or in chains
- **Spores are oval, subterminal, bulging**
- Motile with 4-8 peritrichous flagella
- Form large irregular, glistening colonies with fimbriated border.
- Meat is digested in cooked meat medium with putrid odour.
- Spores are destroyed at 180^{o}C in dry heat for 5-15 min., and in boiling for 5 hours.

III. Clostridium perfringens

★ Gram +ve, obligate anaerobe,

★ Non-motile,

★ **Spore-forming, Spores are oval, central or subterminal, not bulging.**

★ Capsulated

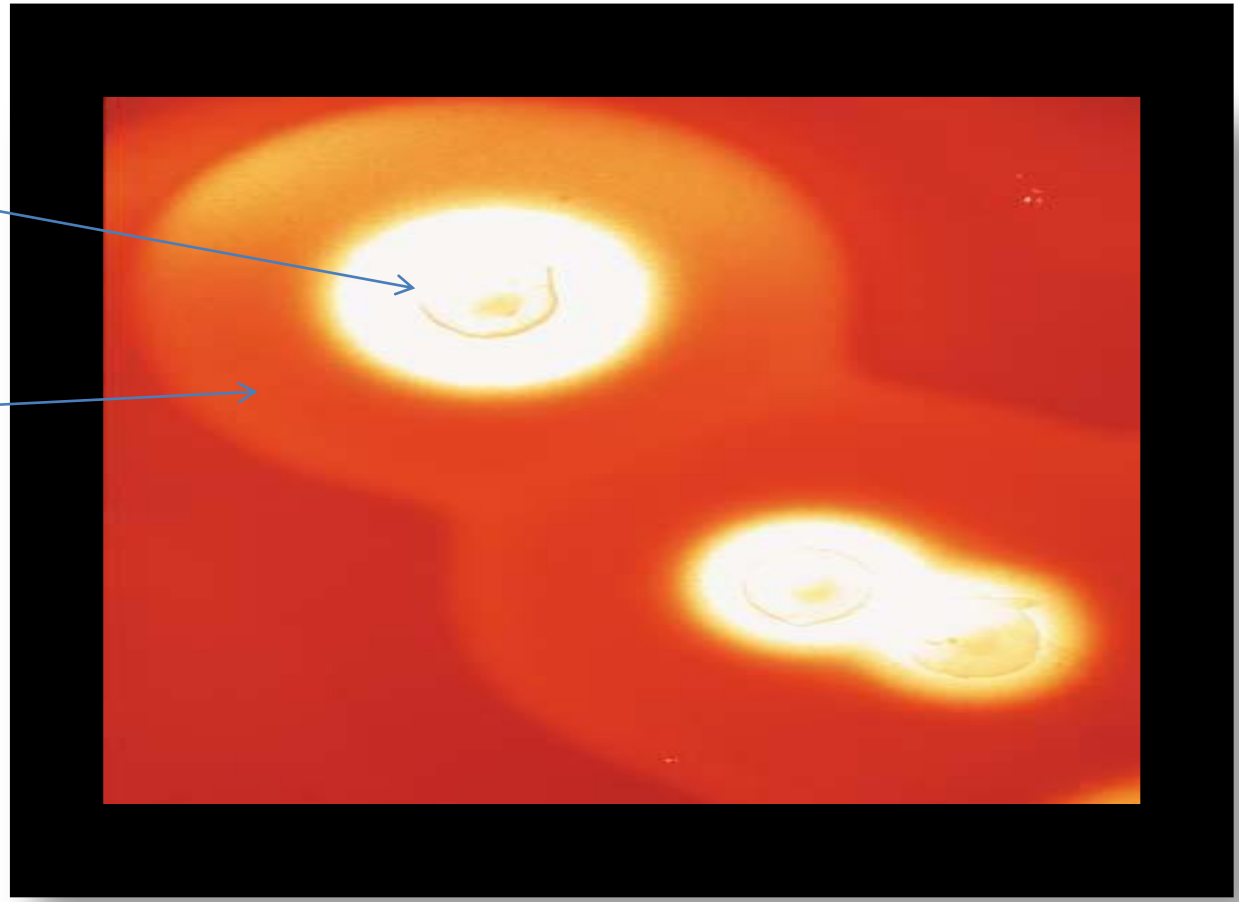
★ Grow best on media containing carbohydrates such as; glucose agar, or glucose blood agar.

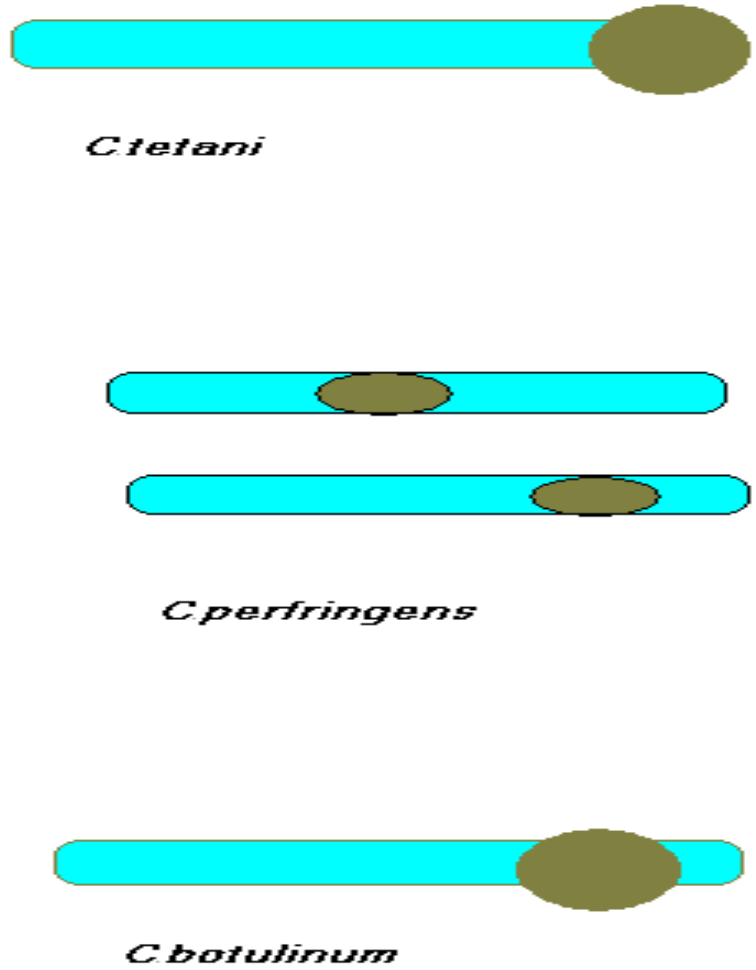
★ Spores of strains of food poisoning resist boiling for 1-3 hours. Other strains can resist boiling for several hours.

★ **Produce double zone inhibition {narrow zone of complete hemolysis (theta toxin), while α- toxin (lecithinase) produce a wide zone of hemolysis}.**

★ **C. *perfringens cause gas gangrene and food poisoning***

NOTE : *C. perfringens* produce double zone of hemolysis





Types of spores

IV. *Clostridium difficile*

- which overgrows during antibiotic therapy, may lead to *pseudomembrane colitis.*

Laboratory diagnostic tests

Clostridium spp. could be divided into:

1. **Saccharolytic organisms**; ***C. perfringens***, ferments carbohydrates, produces acid and gas
2. **Proteolytic organisms**; ***C. tetani***, digest proteins with blackening and bad smell production.
3. **Mixed saccharolytic & proteolytic**; certain groups of ***C. botulinum***.

Specimen: Wound, food, stool.

1. Gram stain: G+ve rods with spore site according to spp

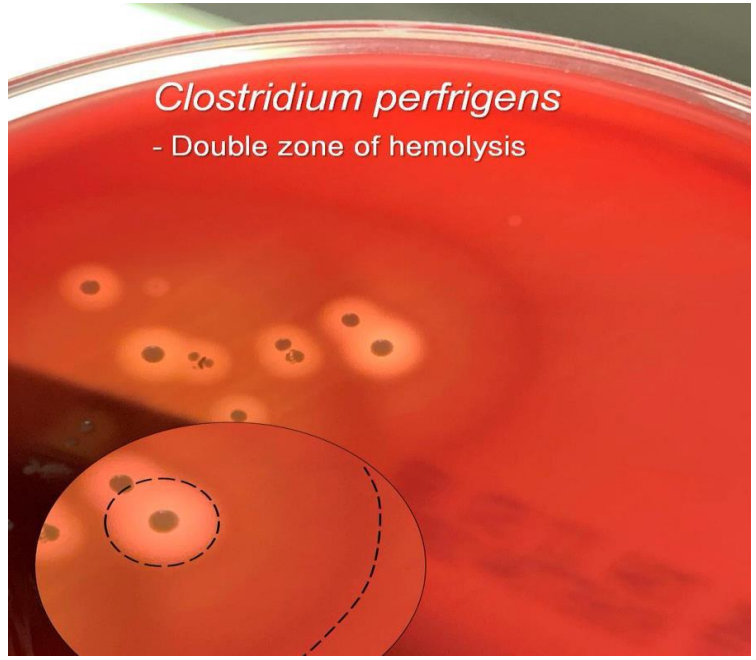
2. Culture: Cultures are incubated under anaerobic conditions.

a. Blood agar:

- Most clostridia produce flat, gray colonies.
- They are also usually hemolytic.
- ***Clostridium perfringens*** produces raised, entire colonies with double zone hemolysis, the complete zone round the colony is due to theta toxin, and the wider zone around it is due to alpha toxin (lecithinase).

Micro & Macroscopic C. perfringens

Gram stain: G+ve rods



***C. perfringens* on blood agar with double zone of hemolysis**

b. Robertson's medium (cooked meat medium):

Cooked Meat Medium provides a favorable environment for the growth of anaerobes, since the muscle protein in the heart tissue is a source of amino acids and other nutrients.

The sulfhydryl groups, which exert the reducing effect, are more available in denatured protein; therefore, the meat particles are cooked for use in the medium.

Cooked Meat Medium is still widely used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes.

- On Robertson's cooked meat medium → blackening of meat will be observed

The anaerobic conditions can be provided by:

(Growth & gas produced are evident).
Gas produced lifted the cooked meat particles to the surface.

Cooked Meat Medium Tubes

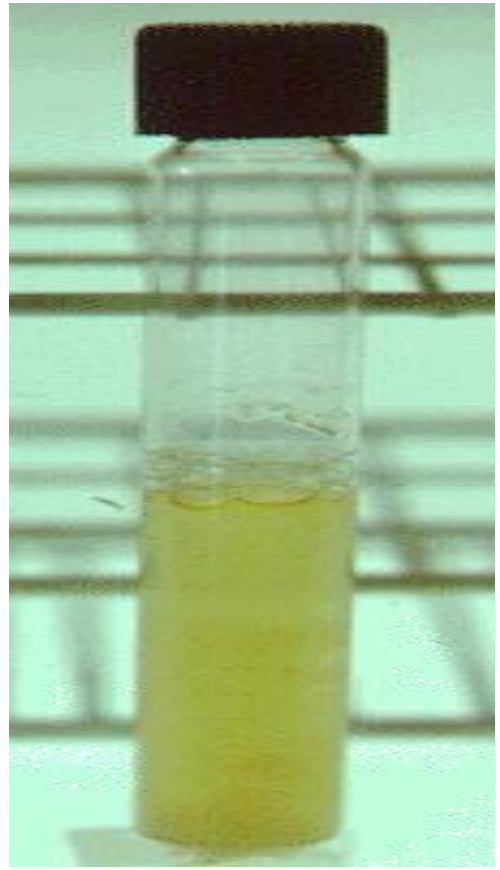
Reaction on Cooked Meat Medium

- Saccharolytic reaction
  - It causes fermentation of glycogen of muscles
  - Production of acid and gas
  - Meat particles remain intact
  - e.g *C. Perfergines*

- Proteolytic Reaction
  - It causes digestion of meat particles with the production of H2S and NH3
  - Formation of black, foul smelling due to sulfur compounds
  - e.g *C. tetani*.

c. Brewer Thioglycollate Medium :

- Contains highly nutritious proteose peptone and beef infusion which support the growth of fastidious bacteria.
- Sodium thioglycollate helps to create anaerobic conditions.
- **Methylene blue indicates** oxygen content of the medium by exhibiting a bluish-green color of the medium in the presence of oxygen.
- Brewer's anaerobic jar is used for growing bacteria under anaerobic condition.



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

The anaerobic conditions can be provided by:

Anaerobic Jar

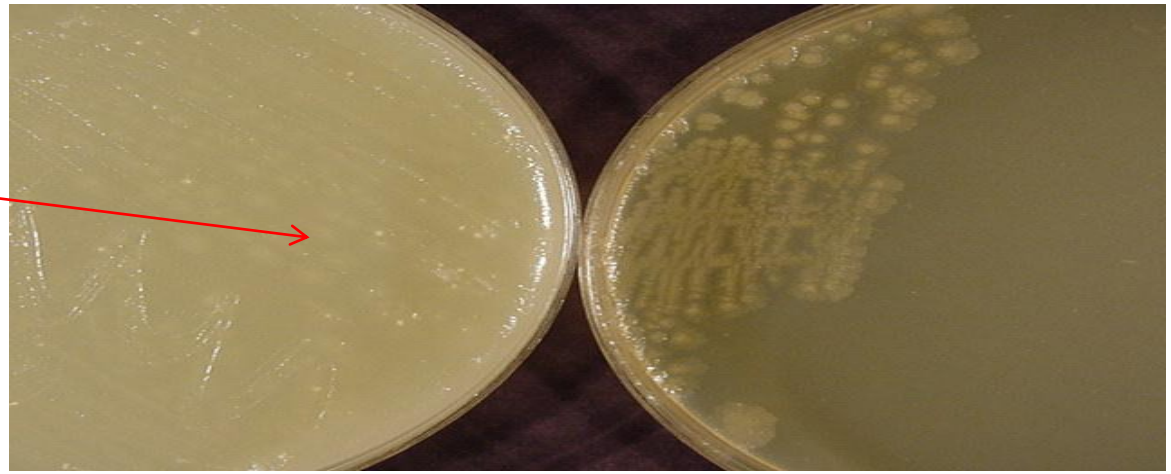
d. Egg yolk agar medium

Definitive **diagnostic** test for ***C. perfringens***

with *C. perfringens* growth

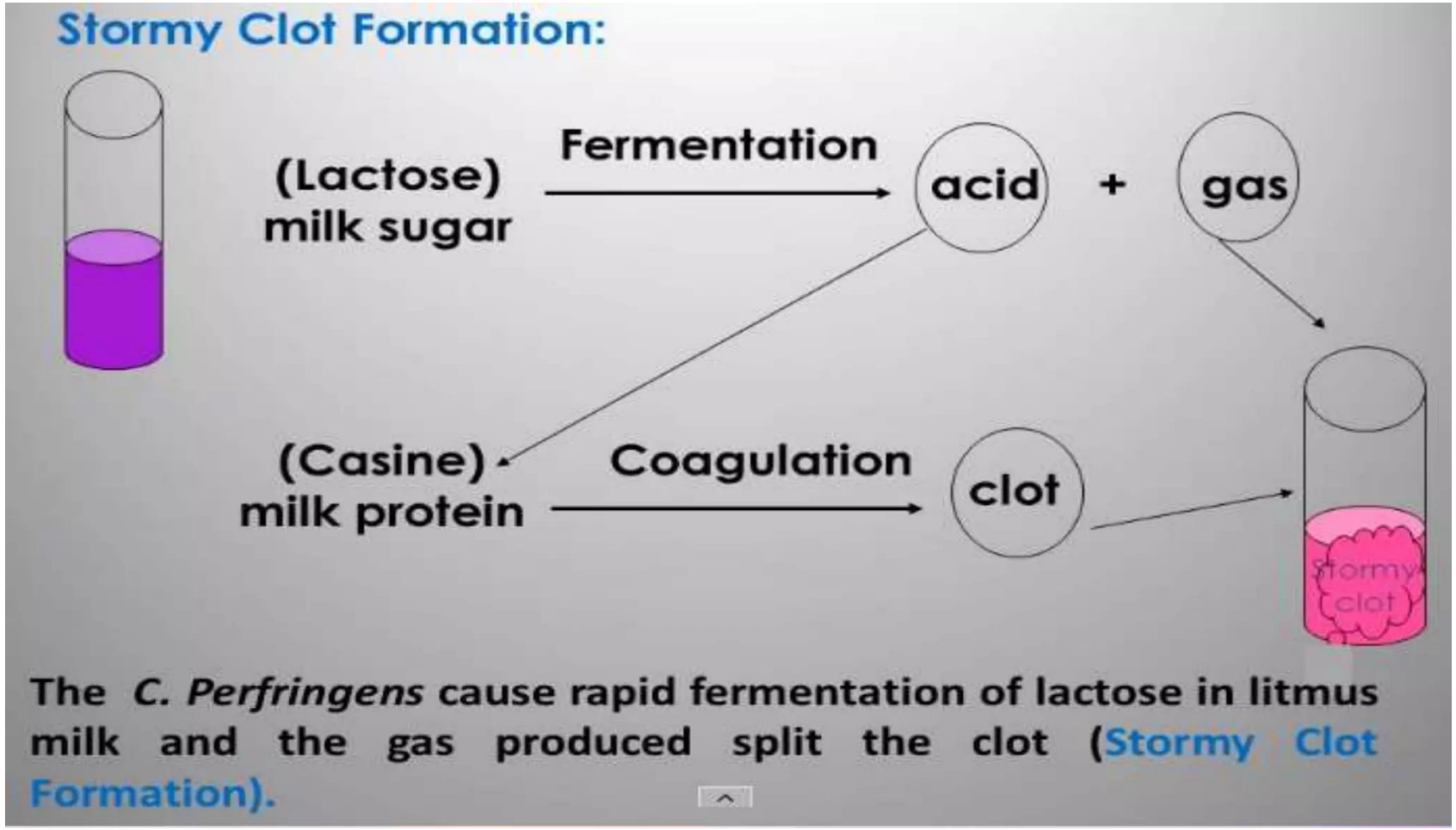
White opaque insoluble precipitation around colonies due to lecithinase activity

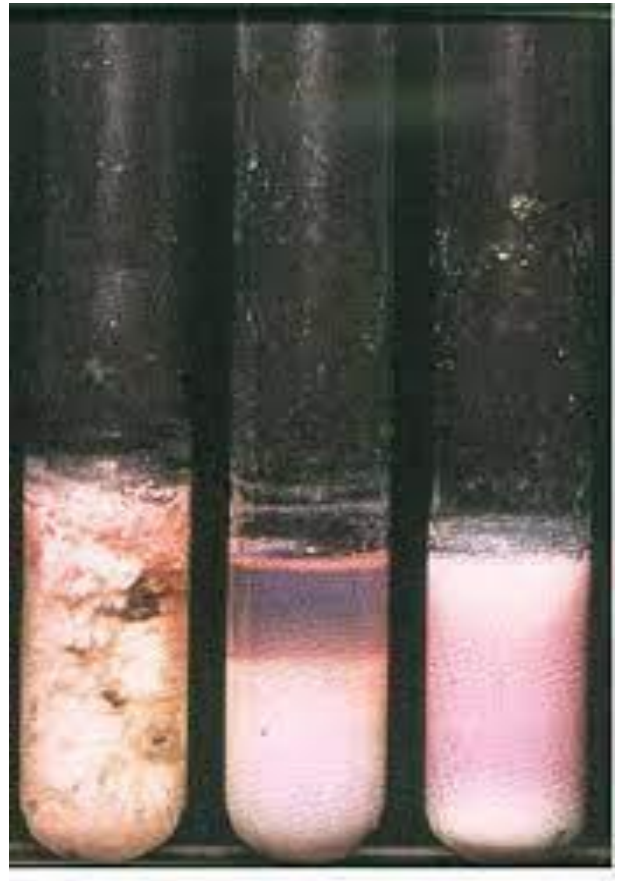
C. perfringens



3. Biochemical tests:

a. Stormy fermentation





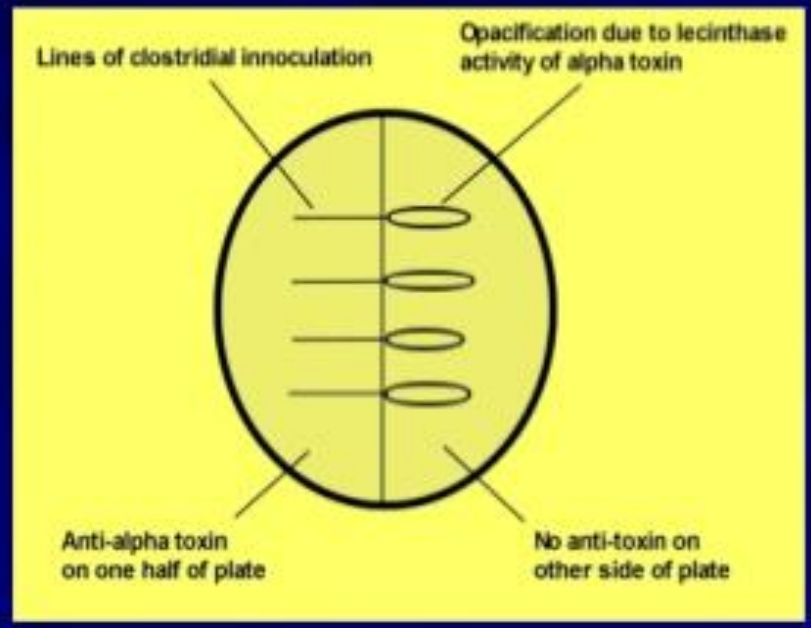
Stormy fermentation

b. Nagler's reaction for the detection of lecithinase activity

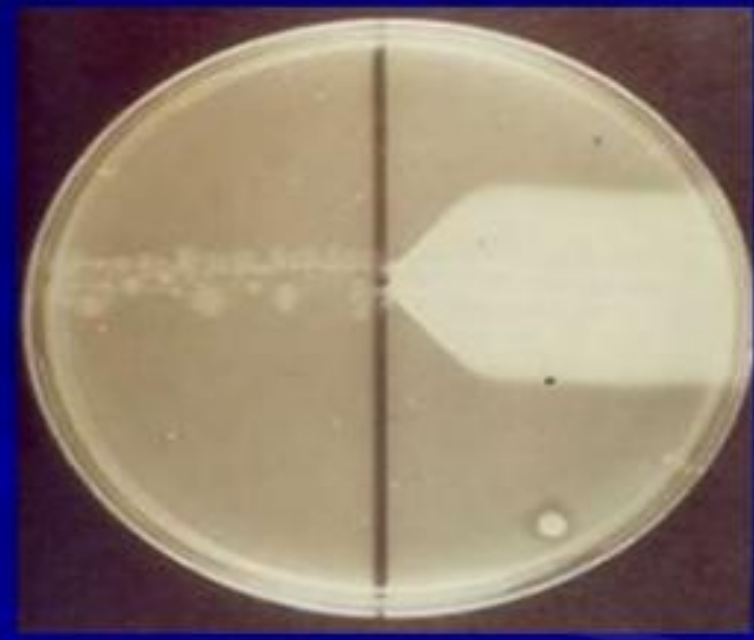
Nagler's reaction is related to the detection of lecithinase activity (alpha toxin of ***Clostridium perfringens)***. Nagler's reaction is a test used to identify bacteria that produce the enzyme lecithinase, which hydrolyzes lecithin, a phospholipid found in egg yolk.

- An egg-yolk plate is divided into two halves. Over one half is spread with a specific antitoxin.
- The culture to be tested, together with a positive control is streaked across the plate, going from the untreated area of the plate to the area that is covered with antitoxin.
- The culture is then incubated anaerobically.
- Following incubation, lecithinase activity, caused by the action of the ***Clostridium perfringens*** a-toxin is seen as a precipitate in the medium on the side of the plate that did not receive the antitoxin, but not on the side that was treated.

Nagler Reaction



Procedure of Nagler Reaction



Positive Nagler Reaction

6. Serological test:

- C. difficil → Toxin in stool by ELISA
- C. tetani → Toxin in serum by ELISA
- C. botulinum → Toxin in serum by ELISA

Thank you