460 MBIO
Medical Bacteriology

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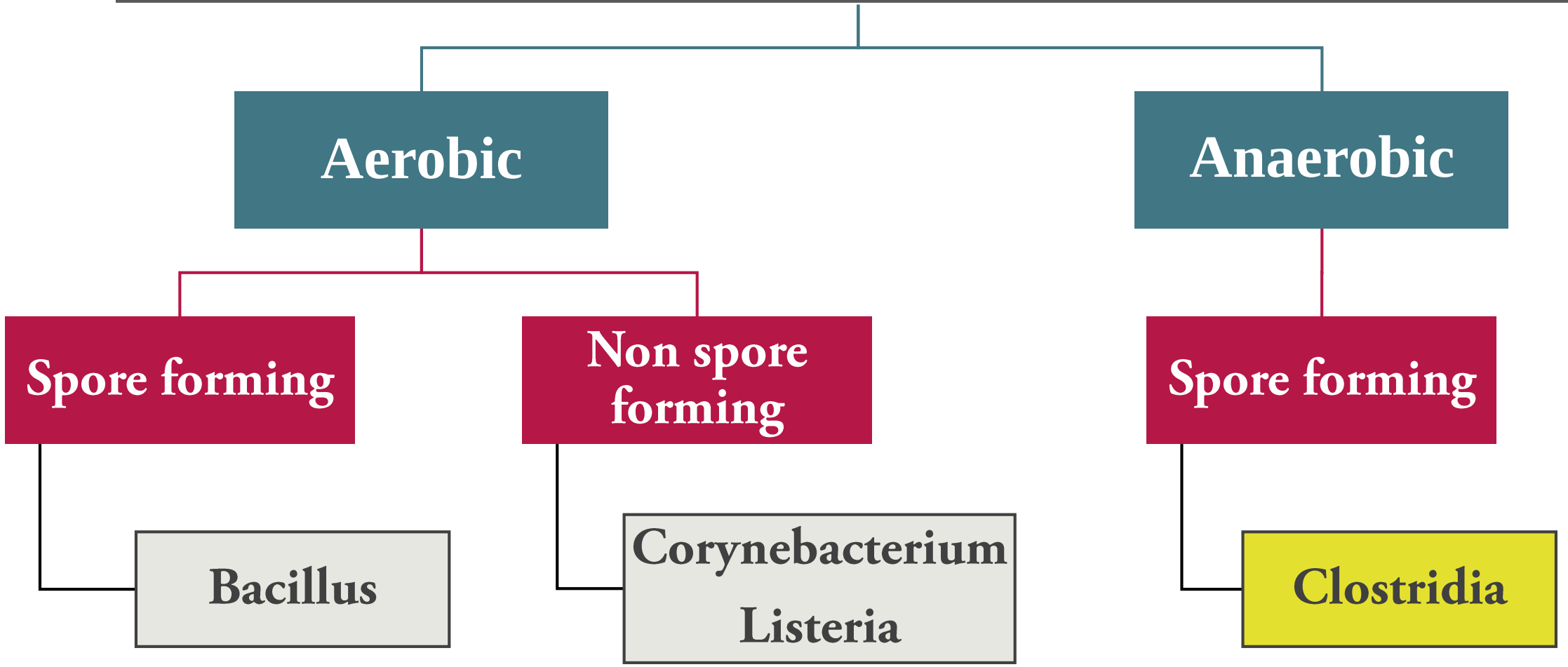
2017

Lab 4
Gram Positive Group
(Clostridia)

Gram positive bacilli divided into two groups according to Oxygen requirements :



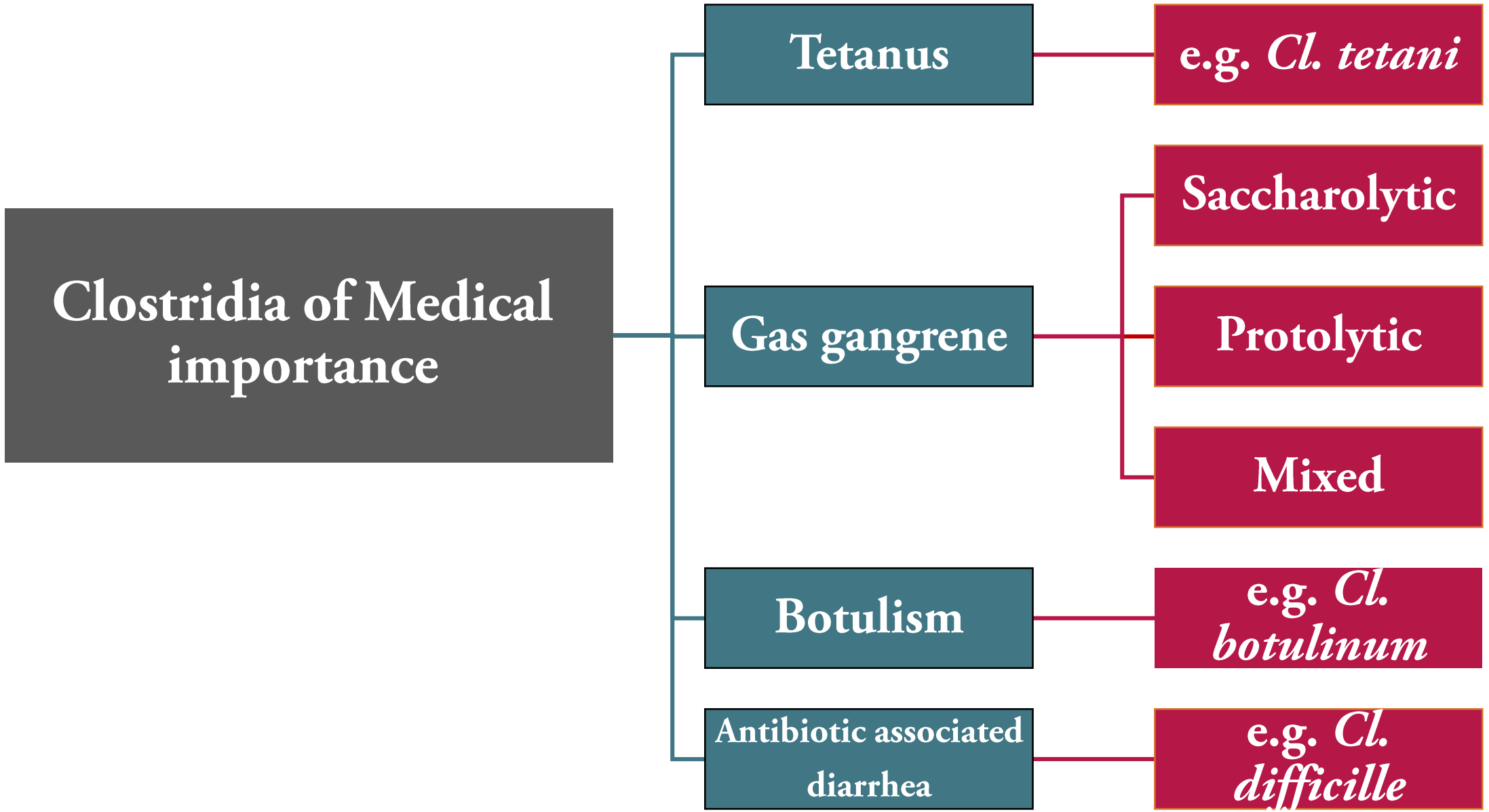
❖ General Characters of Clostridia :

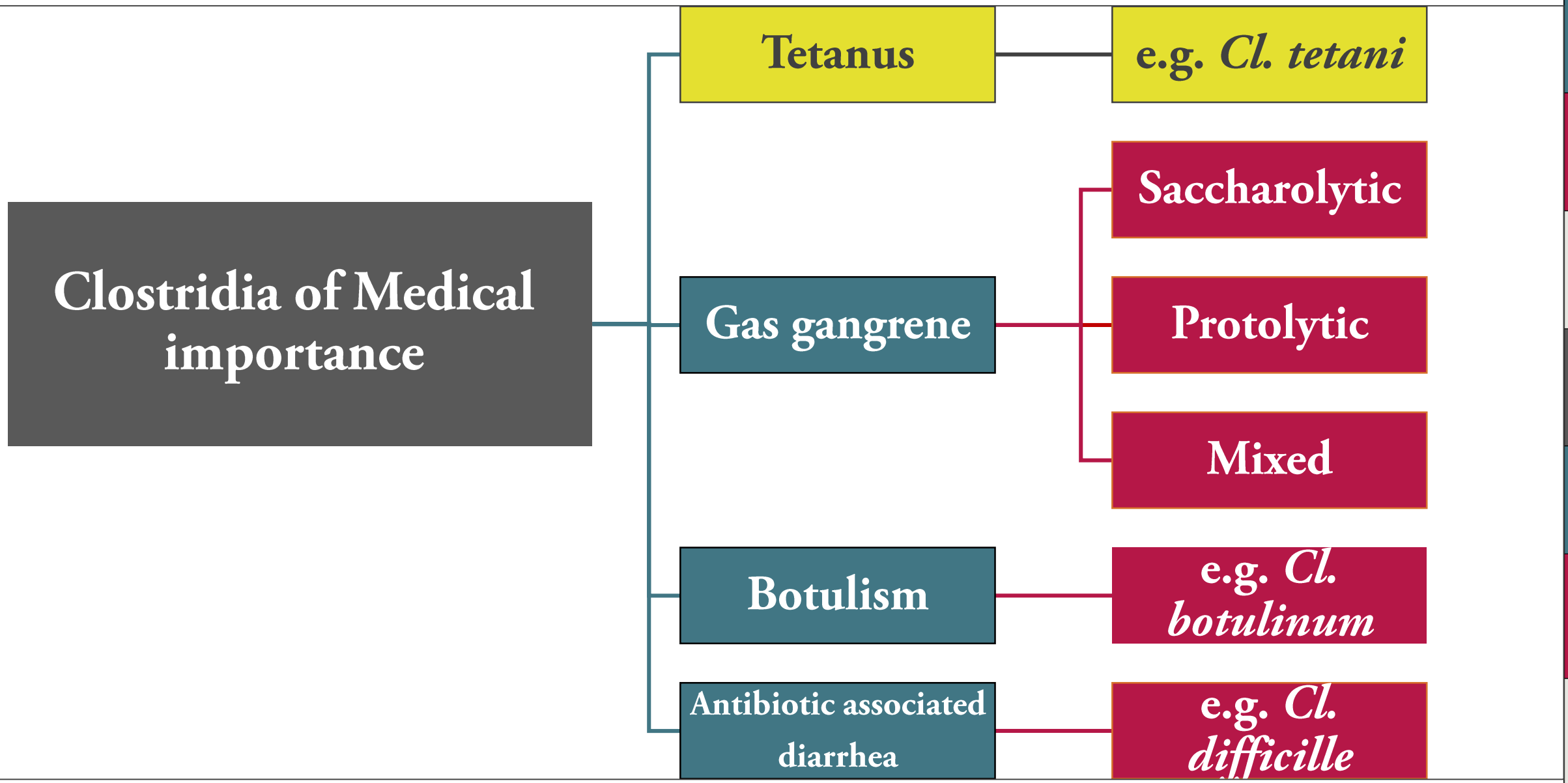
- Large gram positive bacilli.
- Straight or slightly curved rods with slightly rounded ends.
- Spore forming – Anaerobic – Saprophytes.

❖ General Characters of Clostridia :

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


Clostridia of Medical importance
Tetanus
e.g. Cl. tetani
Gas gangrene
Saccharolytic
Protolytic
Mixed
Botulism
e.g. Cl. botulinum
Antibiotic associated diarrhea
e.g. Cl. difficille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❖ 1st : Tetanus (*Clostridium tetani*) :

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

Clostridium tetani Toxins

Tetanolysin — Caused lysis of RBCs

Tetanospasmin — It is a 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 :**

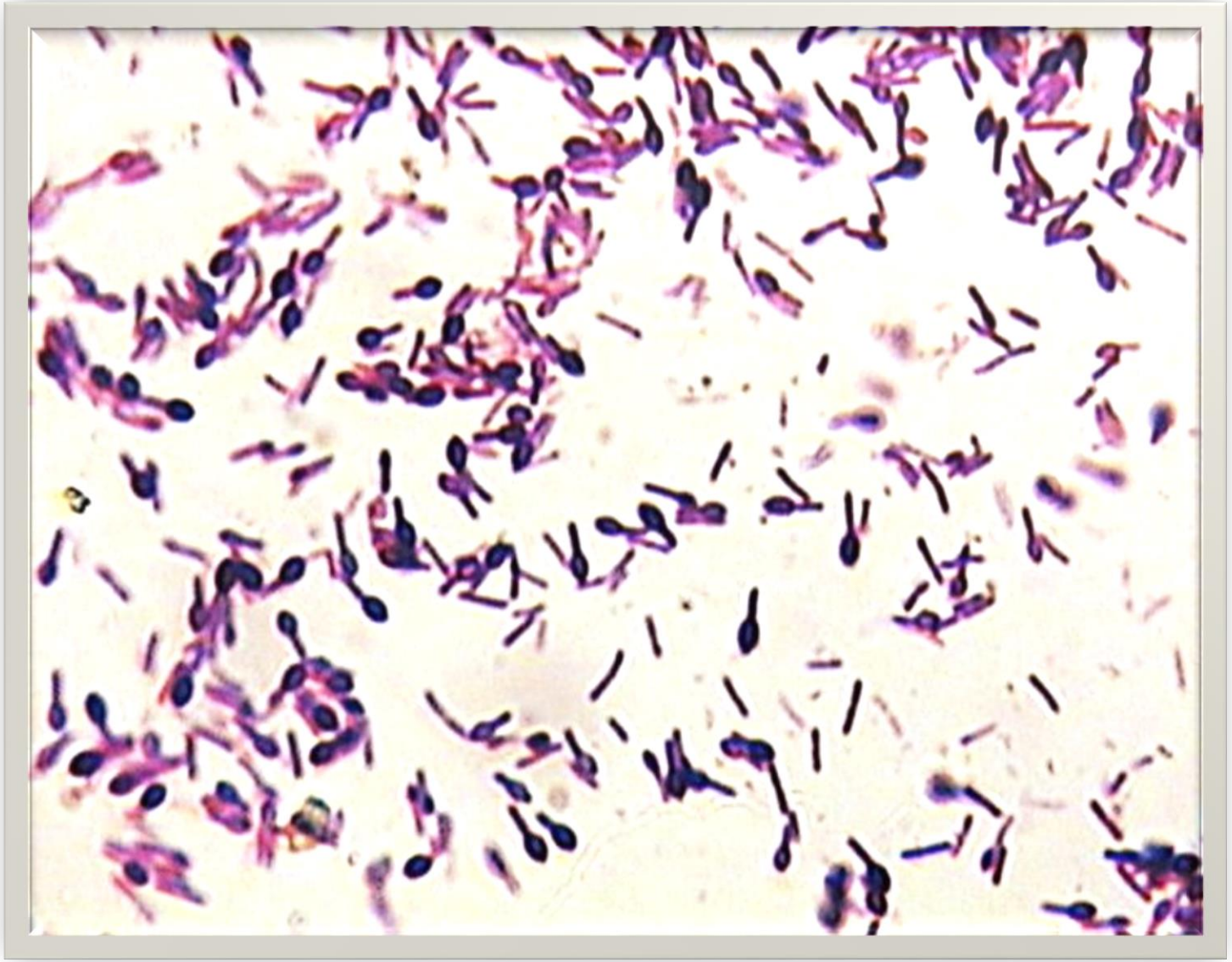
  - Wound exudates using capillary tube.

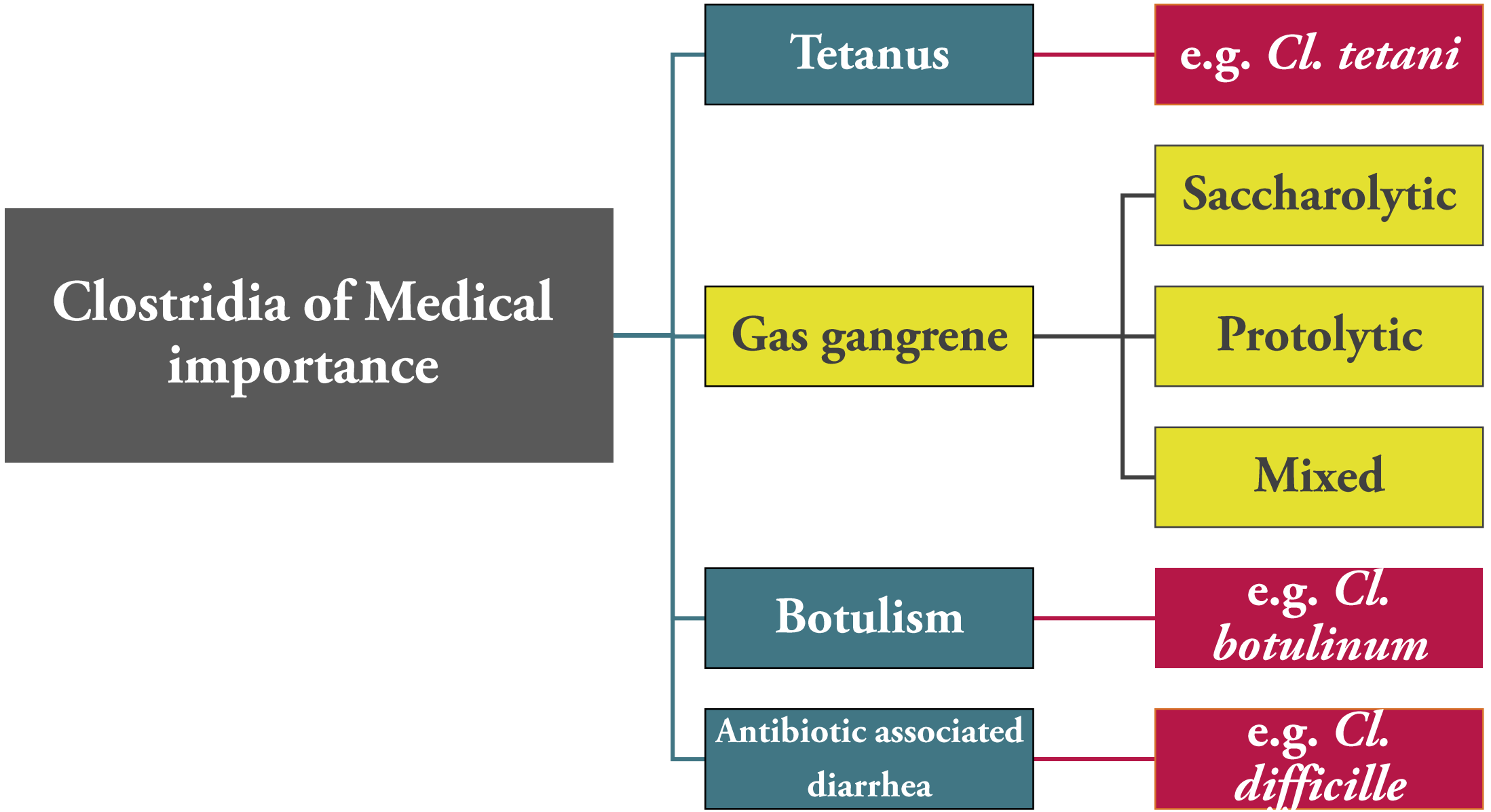
- **Culture :**

  - On blood agar and incubated anaerobically.

- **Gram stain :**

  - *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance.

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Clostridia causing Gas Gangrene

	Description	Example
Saccharolytic	▪ Ferment carbohydrates, which produce acid and gas.	▪ *Cl. Perfringens* ▪ *Cl. septicum*
Proteolytic	▪ Digest proteins which result a production of blackening and bad smell.	*Cl. sporogenes*
Mixed	Mixed saccharolytic & proteolytic	*Cl. histolyticum*

❖ 2nd : *Clostridium perfringens* :

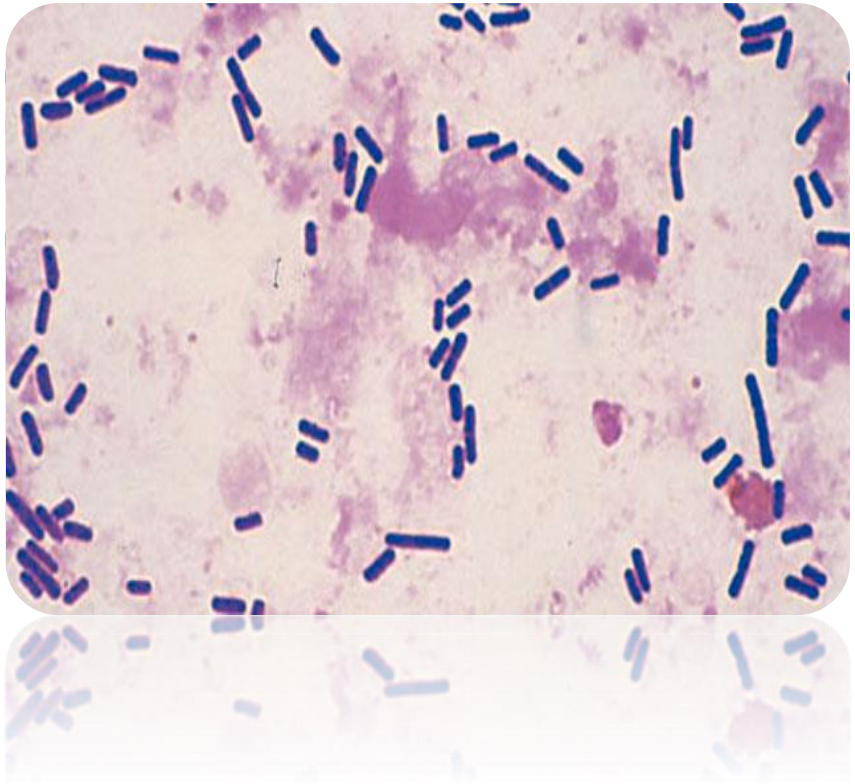
- Large Gram-positive bacilli with stubby ends.
- Does not sporulate on ordinary media.
- Capsulated and non motile.
- Grown quickly on selective media.

Clostridium perfringens Toxins

- **α – toxin Lecithinase**
  - It is the most important toxin, It can lysis each of RBCs, platelets, leucocytes and endothelial cells.
- **β - toxin**
  - It is responsible for necrotic lesions.
- **Enterotoxin**
  - It is heat labile toxin produced in colon and caused food poisoning.

❖ Laboratory diagnosis of *Cl. perfringens* :

- **Specimen.**
- **Morphology :**
  - ➢ Macroscopical (Cultural characteristics)
  - ➢ Microscopical (Gram stain)
- **Biochemical Test.**



1st : Specimen :

- It should be taken from :

- **Histological specimen.**
- **Wound exudates :** specimens taken from the deeper areas of the wound where the infection seems to be most pronounced.

2nd : Morphology, Macroscopical

Cooked Meat medium	Blood Agar
Blackening of meat will observed with the production of H_2S and NH_3	Appearance of double hemolysis, β and α – hemolytic.

2nd: Morphology, Microscopical :

- **Gram stain**

- Gram positive bacilli, non motile and capsulated.
- The spore is oval, sub-terminal & non bulging.
- Spores are rarely observed.

3rd : Biochemical test

- ***Cl. perfringnes* characterized by :**

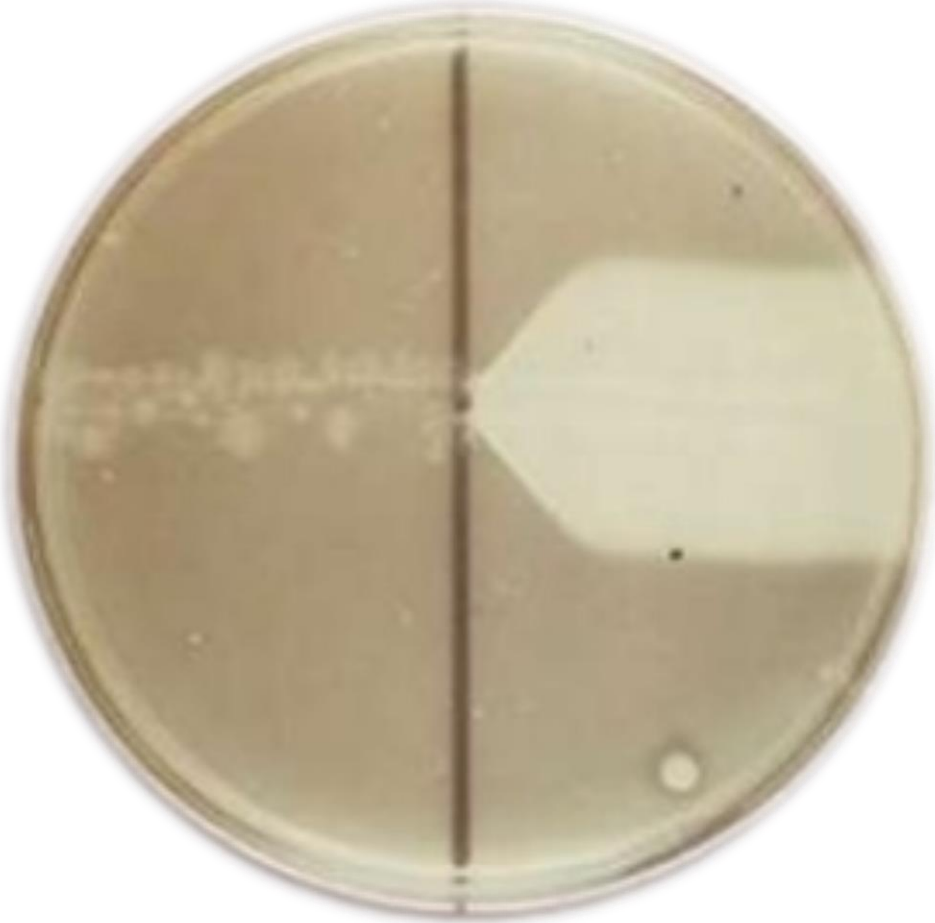
- It ferments many carbohydrates with acid & gas.

- It acidified litmus milk with stormy clot production.

- **Nagler reaction** is positive.

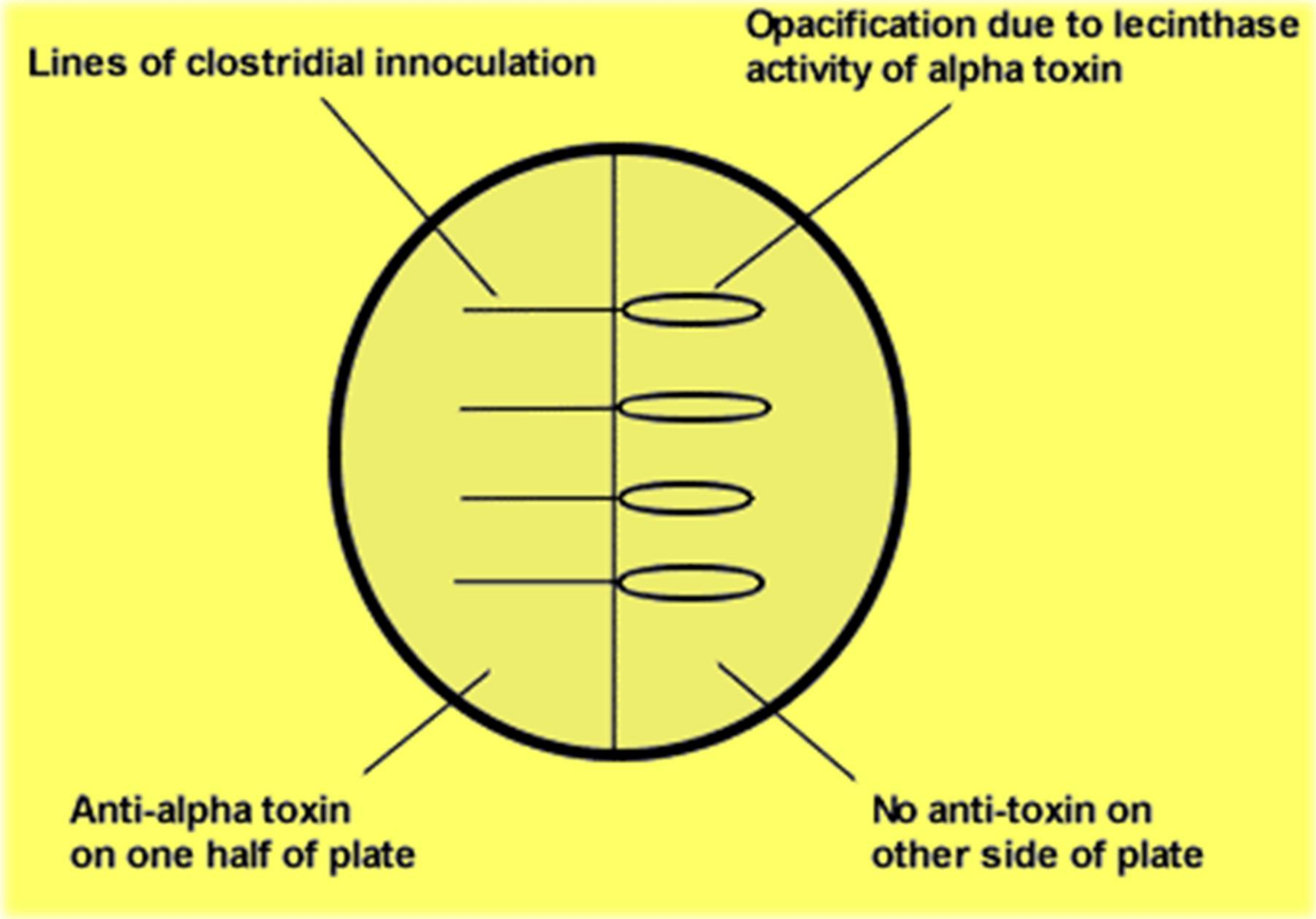
❖ Nagler's Reaction

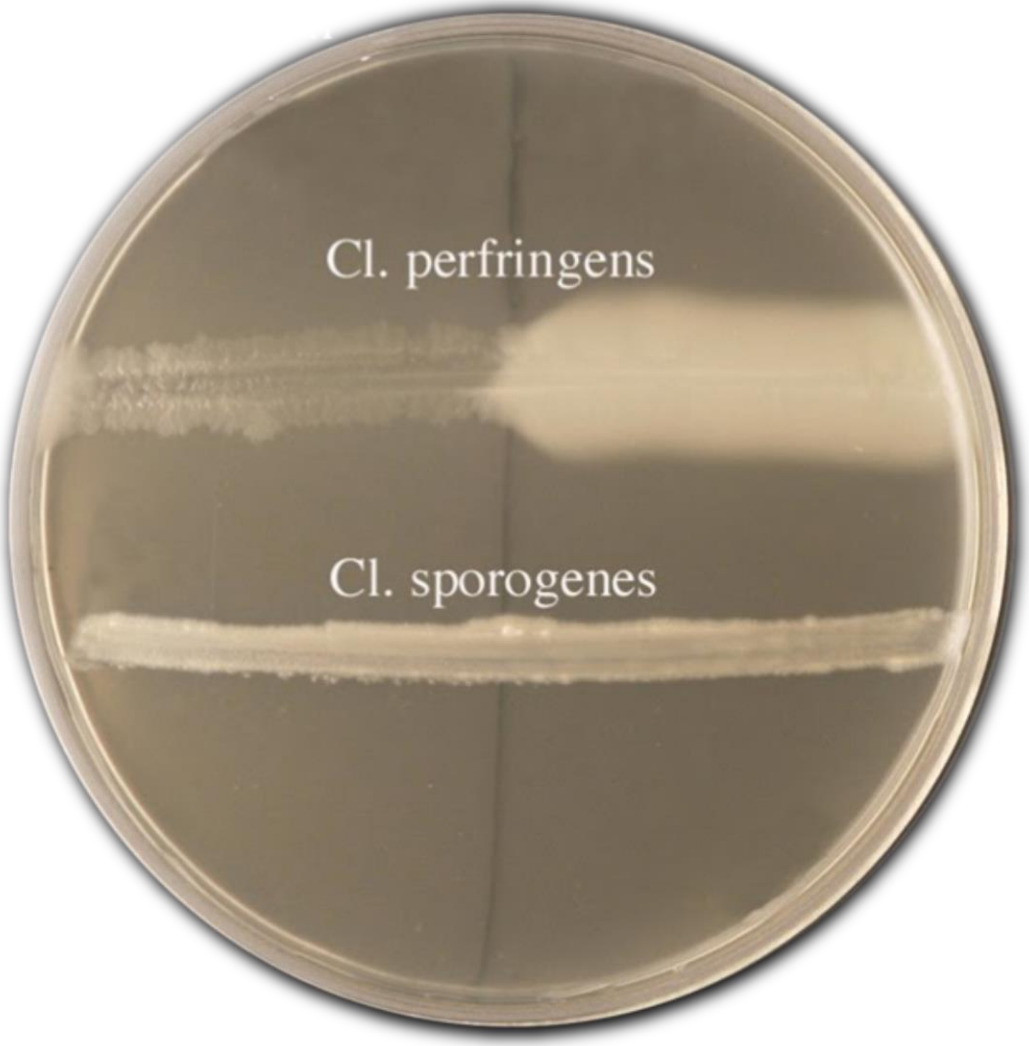
- **Principle :**
  - It is toxin/antitoxin reaction.
  - This test is done to detect the lecithinase activity (α-toxin; phospholipase) which hydrolyzes phospholipid in egg yolk agar causing a turbidity.

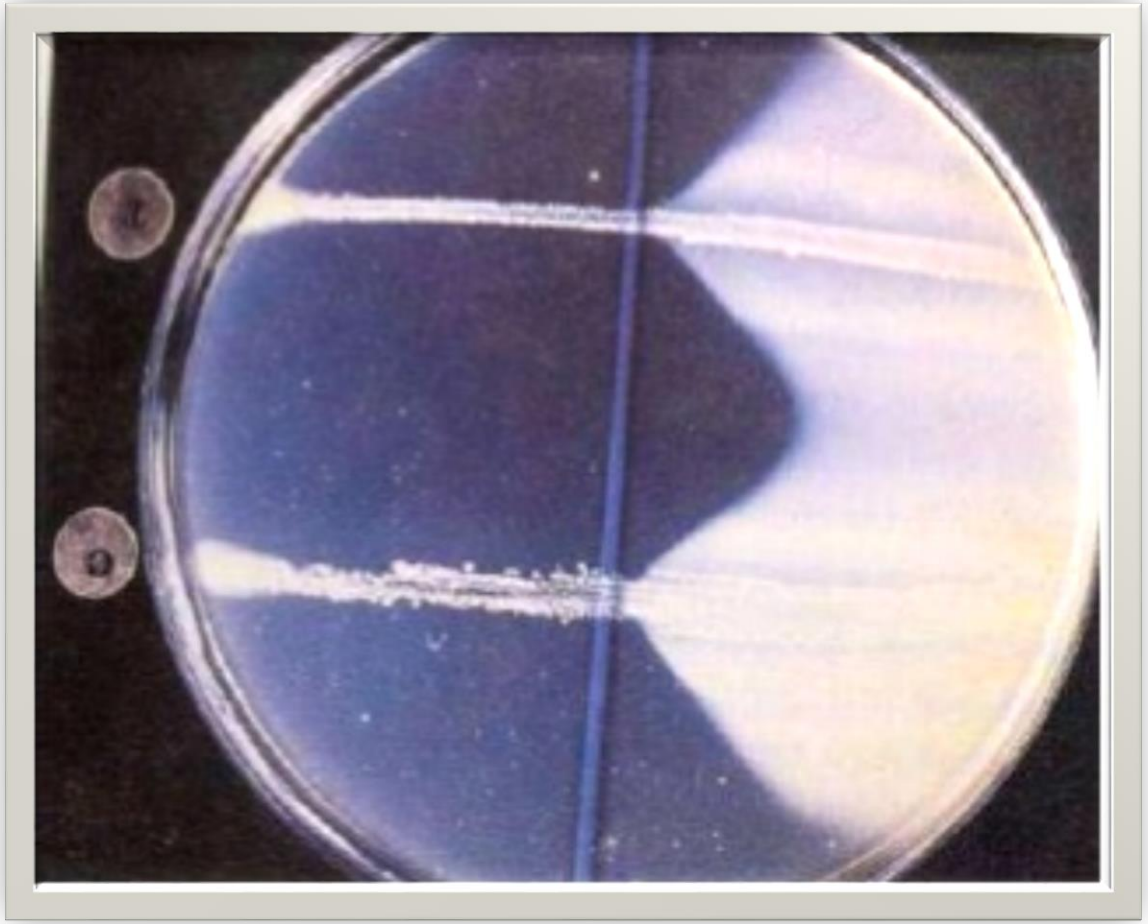


Method and Results

Procedure	▪ Inoculated on the medium containing human serum or egg yolk (contains lecithin) ▪ Incubate the plate anaerobically at 37℃ for 24 hrs.
Result	▪ 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 antitoxin is present on the medium

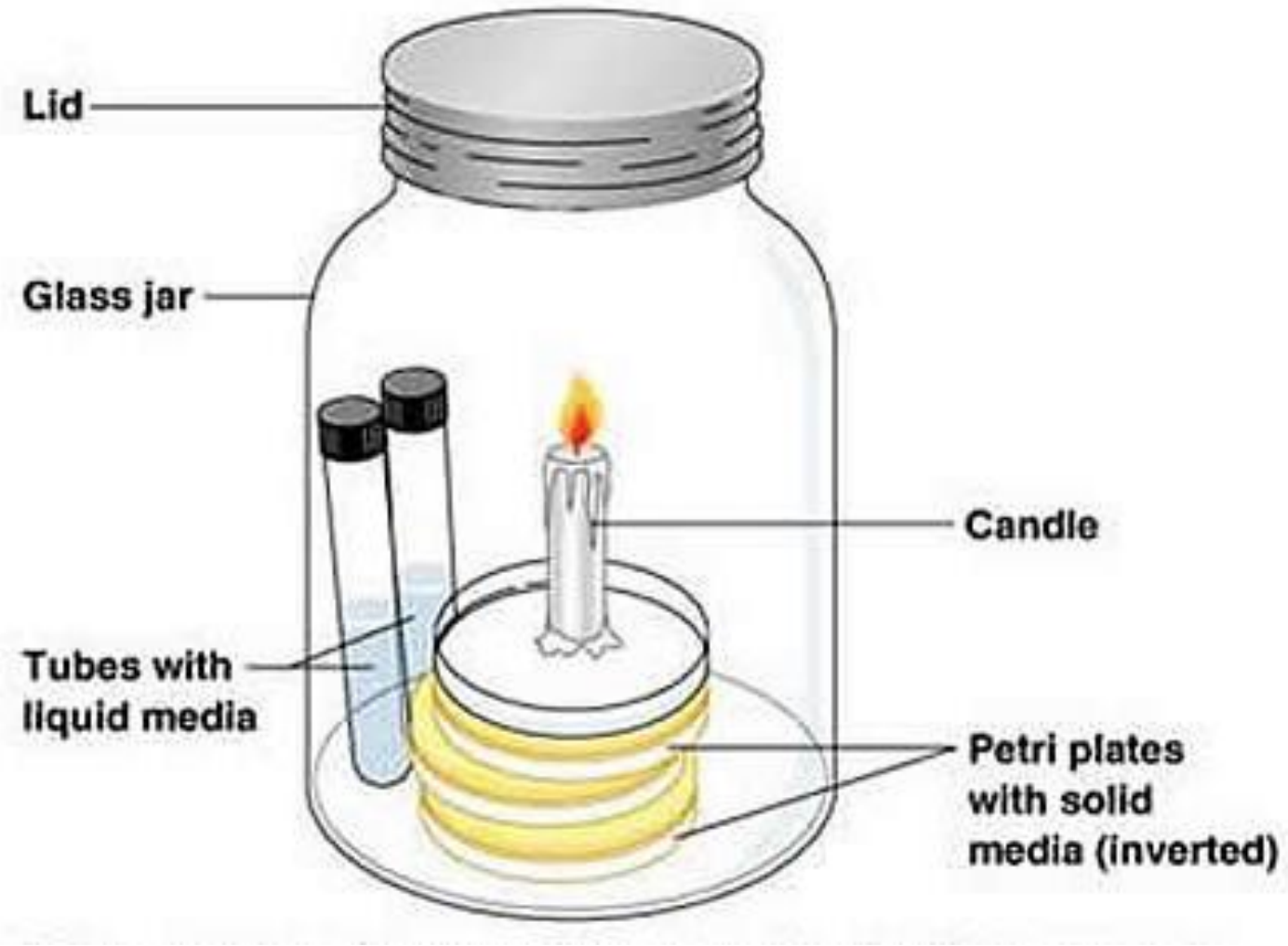

Lines of clostridial innoculation
Opacification due to lecinthase activity of alpha toxin
Anti-alpha toxin on one half of plate
No anti-toxin on other side of plate


Cl. perfringens
Cl. sporogenes



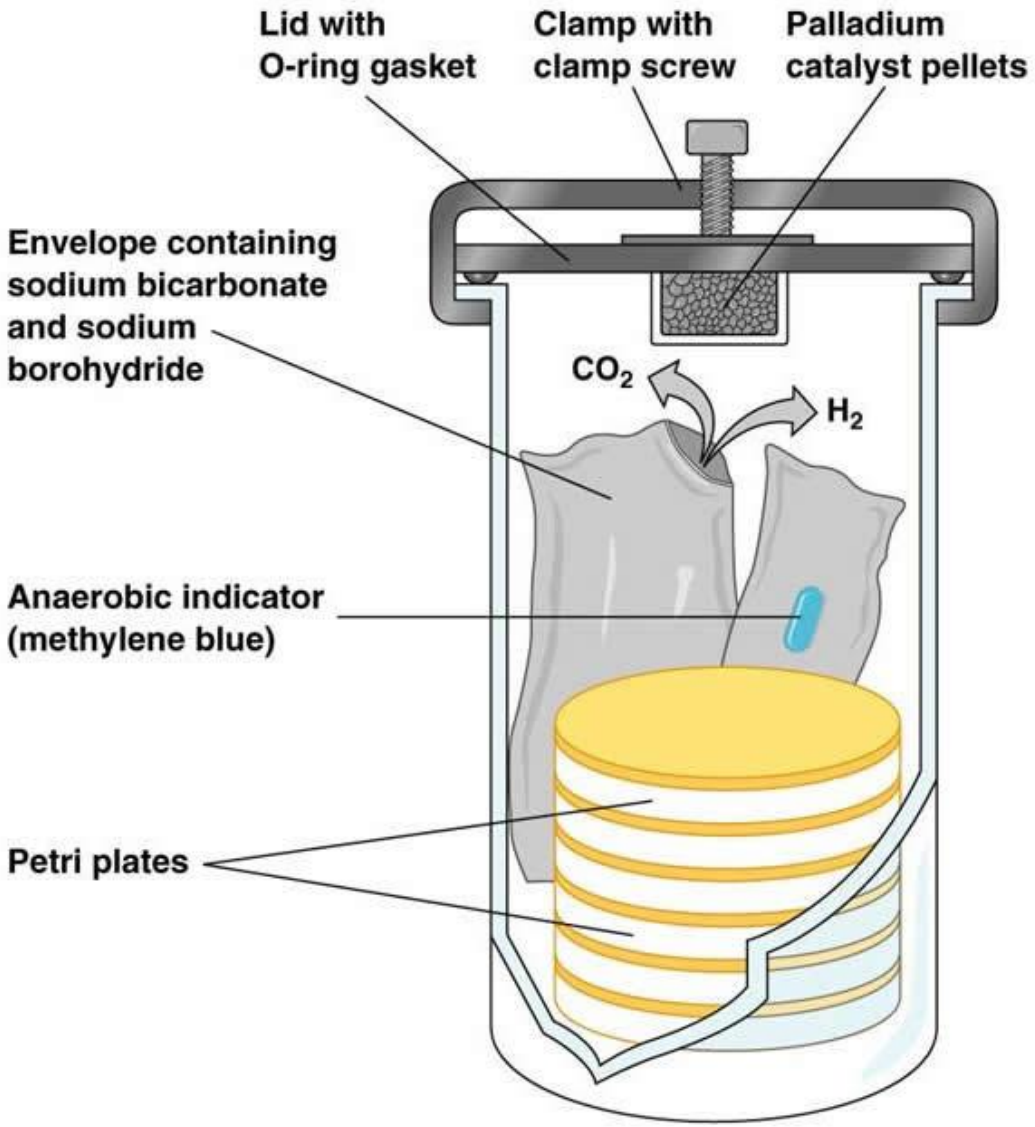
❖ Anaerobic Cultivation

- Removal of oxygen & replacing it with inert gas.
- Anaerobic cultivation could be by use :
  - ➢ Anaerobic jar.
  - ➢ Candle jar.
  - ➢ CO_2 incubator.


Lid
Glass jar
Candle
Tubes with liquid media
Petri plates with solid media (inverted)

Anaerobic Cultivation Method

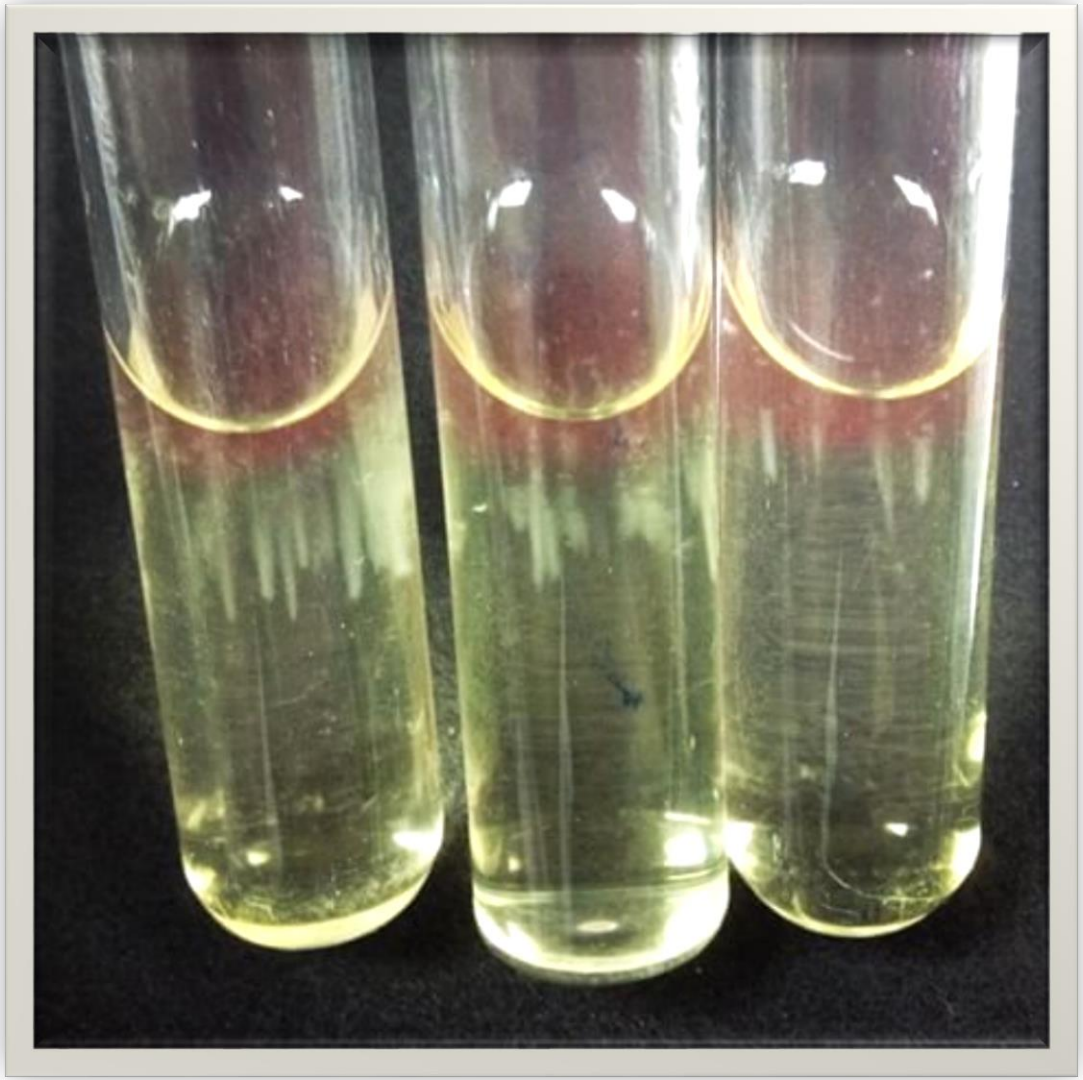
- It is especially plastic jar with a tightly fitted lid.
- Hydrogen is introduced from commercially available hydrogen generators envelop.
- 10 ml of water is added to envelop immediately before placing it in the jar.
- Hydrogen and carbon dioxide will release and react with oxygen in the presence of 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)

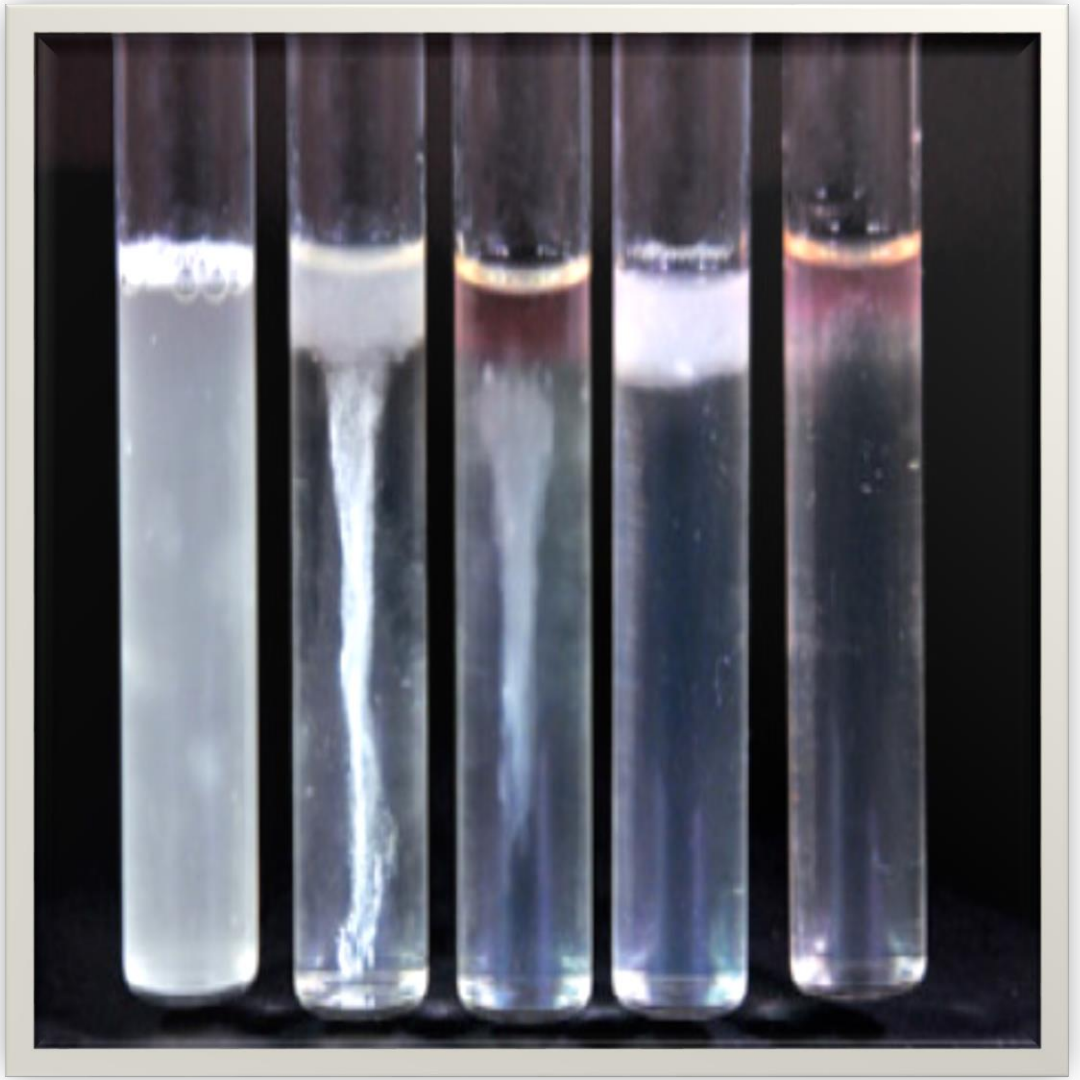

Lid with
O-ring gasket
Clamp with
clamp screw
Palladium
catalyst pellets
Envelope containing
sodium bicarbonate
and sodium
borohydride
CO_2
H_2
Anaerobic indicator
(methylene blue)
Petri plates


BBL CampyPak Plus

Anaerobic Cultivation Medium

	Thioglycolate Broth	Cooked meat Medium
Reducing agent	Sodium thioglycolate	Meat particles contains hematein & glutathione
Indicator	Rezazurin	Blackening
Further component	Low percentage of Agar-Agar to increase viscosity of medium	Meat particles (prepared from heart muscles)
Result	Turn pink from yellow	Depends on the type of lytic





Reaction on Cooked Meat Medium

	Saccharolytic	Protolytic
Effect	It causes fermentation of glycogen of muscles	It causes digestion of meat particles
Production	acid and gas, meat particles remain intact	Formation of black, foul smelling due to sulfur compounds
Example	*Cl. perfergines*	*Cl. botulinum* group I

Any Questions

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