

# Bacteria Classification

**Clostridium spp**

**Lab 4**

# Spore-Forming Gram Positive Bacilli

## Scientific Classification of Clostridium Spp.

Domain: Bacteria Phylum: Firmicutes

Class: Clostridia

Order: Clostridiales

Family: Clostridiaceae

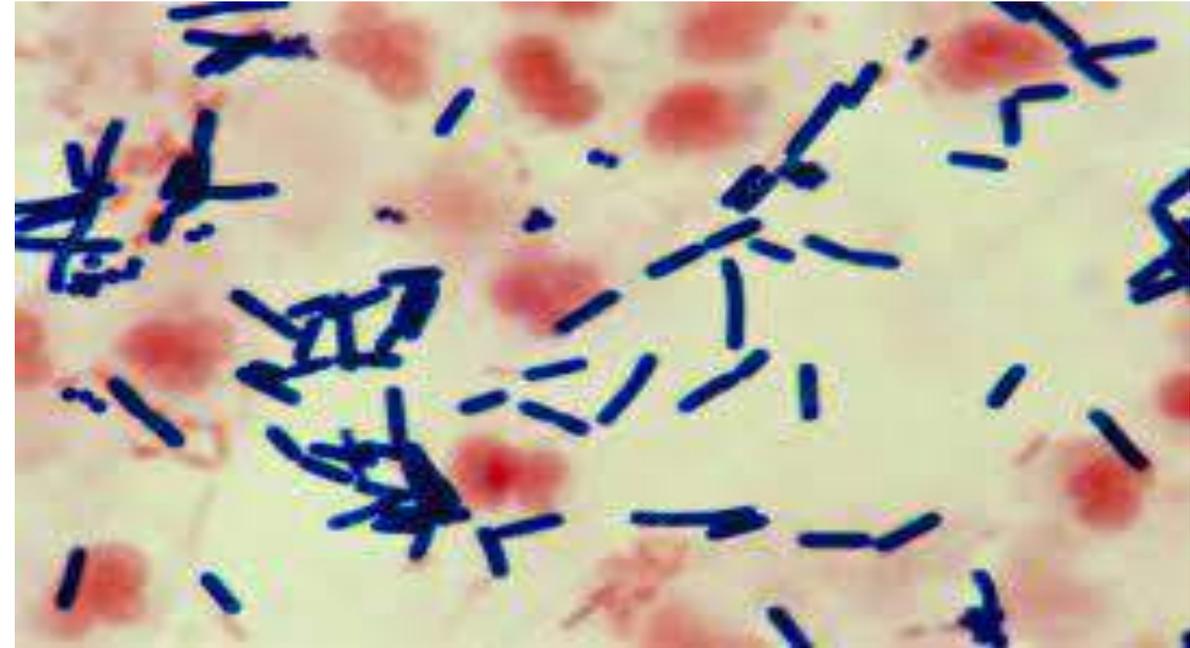
Genus: Clostridium . . . . . include at least 250 species.

Species: *Clostridium perfringens*- gas gangrene

*C. tetani*

*C. botulinum*

*C. difficile*



# GRAM POSITIVE RODS

- ❑ **Bacterial spores** are highly resistant, dormant structures (i.e. no metabolic activity) formed in response to adverse environmental conditions.
- ❑ They help in the survival of the organisms during adverse environmental conditions; they do not have a role in reproduction.

| Spore forming  | Non-spore forming   |
|--|---|
| <ul style="list-style-type: none"><li>• <i>Clostridium</i> spp.</li><li>• <i>Bacillus</i> spp.</li></ul> | <ul style="list-style-type: none"><li>• <i>Corynebacterium</i> spp.</li><li>• <i>Lactobacillus</i> spp.</li><li>• <i>Mycobacterium</i> spp.</li></ul> |

# GENERAL CHARACTERISTICS

- **Gram-positive rods** (at least early in growth), in singles, pairs, or chains.
- Most are obligate anaerobes, but some are **microaerophiles**.
- Produces endospores, but not aerobically; spore shape and position are variable, but usually distend the cell
- Most are catalase-negative and oxidase negative.
- Most are isolated from soil, sewage, or marine sediments.
- Key pathogens are *C. tetani* (tetanus), *C. botulinum* (botulism), *C. perfringens* (food poisoning and gas gangrene), and *C. difficile* (pseudomembranous colitis).



# GENERAL CHARACTERISTICS

- Endospore's **size, shape and location** is used for differentiation
- **Mostly motile** (except *C. perfringens*)
- Required enriched media for growth
- They are **toxigenic** (has the ability to intoxicate a person)
- They are **non-capsulated** except *C. perfringens*
- **Liquefy gelatin** (gelatin liquefaction +ve)
- **Fermentative**



# TAXONOMY

| Rank                                  | Scientific name  |
|---------------------------------------|--|
| Family                                | <ul style="list-style-type: none"><li>• Clostridiaceae</li></ul>   |
| Genus                                 | <ul style="list-style-type: none"><li>• <i>Clostridium</i></li></ul>   |
| Species<br>(medically important spp.) | <ul style="list-style-type: none"><li>• <i>C. tetani</i></li><li>• <i>C. botulinum</i></li><li>• <i>C. perfringens</i></li><li>• <i>C. difficile</i></li></ul> |



# *Clostridium* spp.

## Samples:

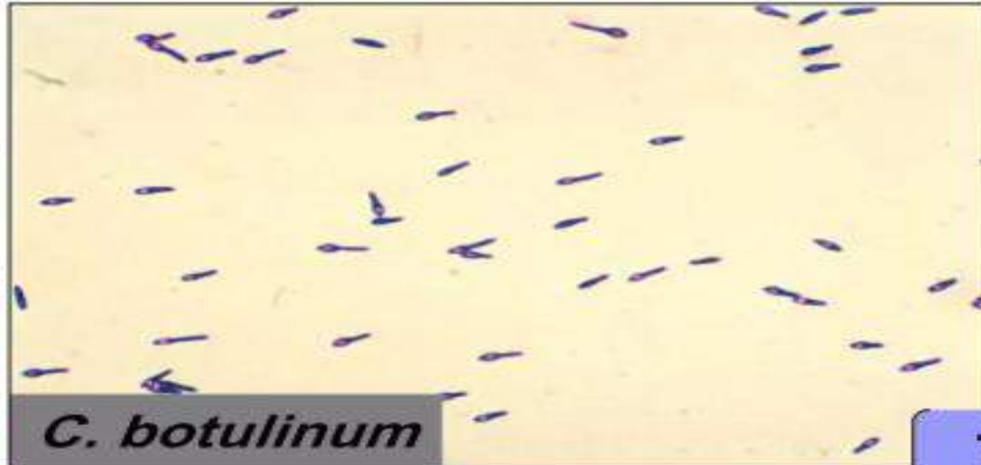
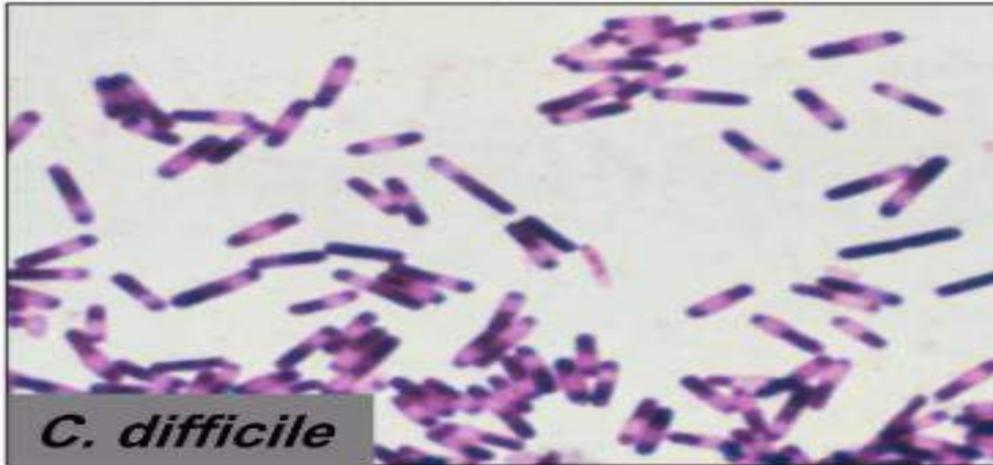
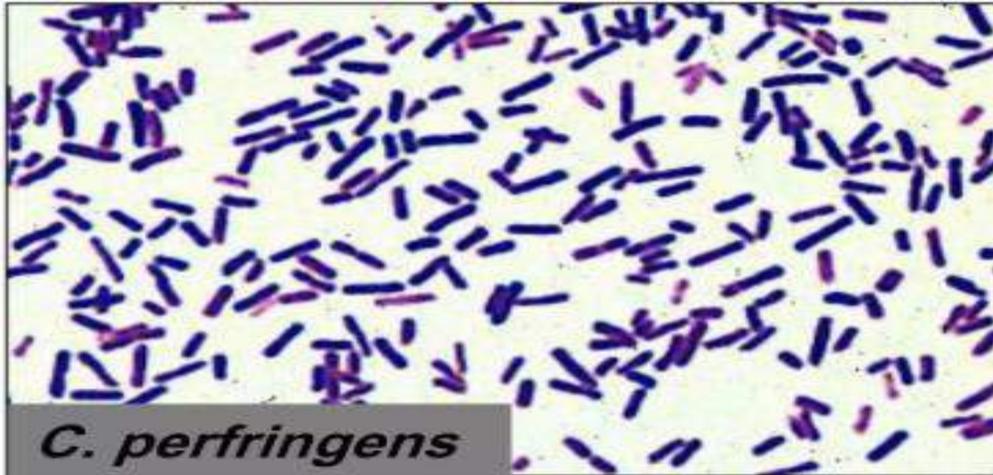
- *C. perfringens*: wound swabs, necrotic tissues, muscle fragments, pus, stool and food (food poisoning)
- *C. difficile*: stool samples (diarrhoea)
- *C. botulinum*: food, faeces or intestinal contents



Diabetic foot

# *Clostridium* spp.

## ■ Microscopic features



# *Clostridium* spp.

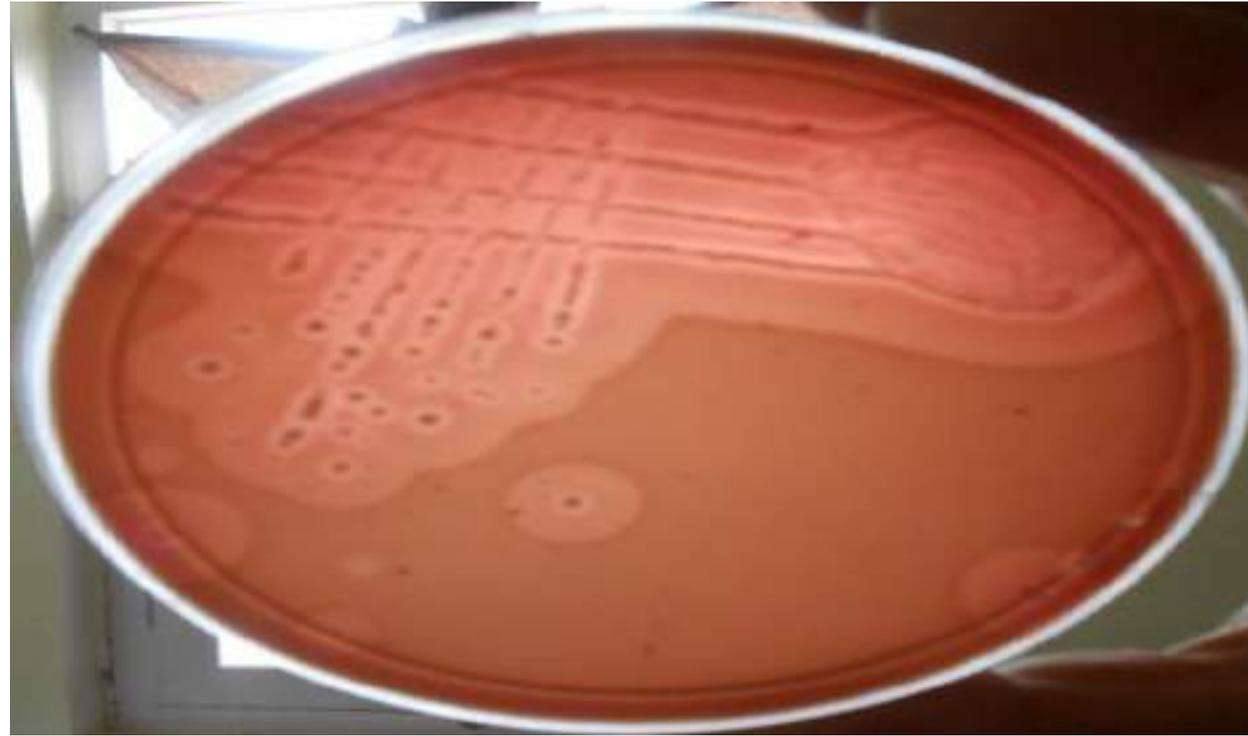
## Microscopic features:

1. *C. perfringens*: Gram +ve, pleomorphic rods, oval, sub-terminal spore and non-motile.
2. *C. tetani*: Gram +ve, long thin rods, terminal spores, (drumstick appearance) and motile.
3. *C. botulinum*: Gram +ve, pleomorphic rods, oval, sub-terminal spore and motile.
4. *C. difficile*: Gram +ve, long thin rods, large oval sub-terminal spores and motile.

- **Double zone of hemolysis** produced by *Clostridium perfringens*: inner zone of complete  $\beta$ -hemolysis and outer zone of partial  $\beta$ -hemolysis.



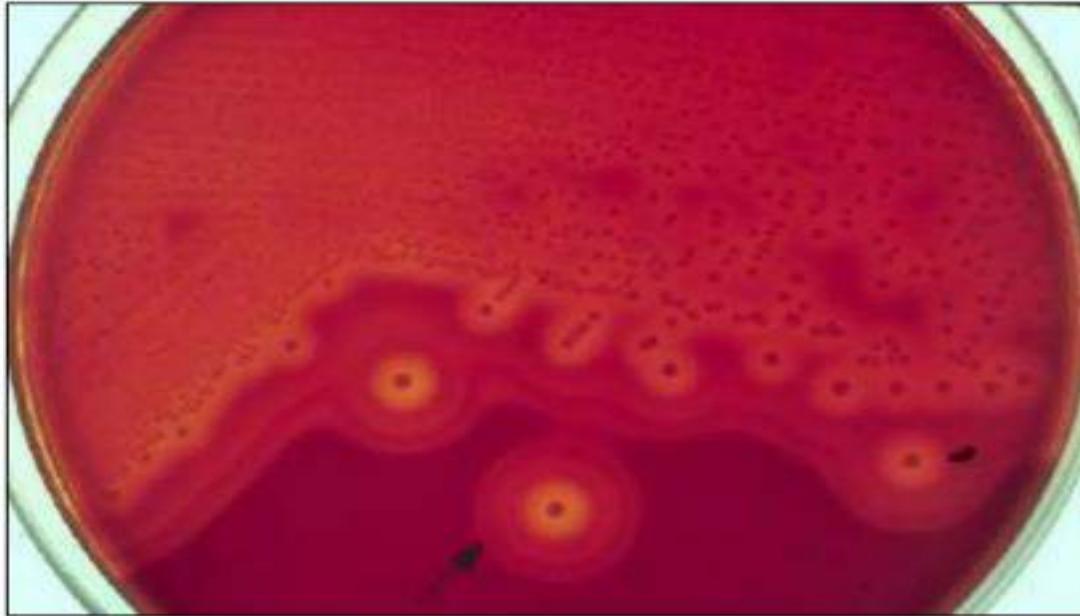
**beta-hemolysis**  
*Streptococcus pyogenes*



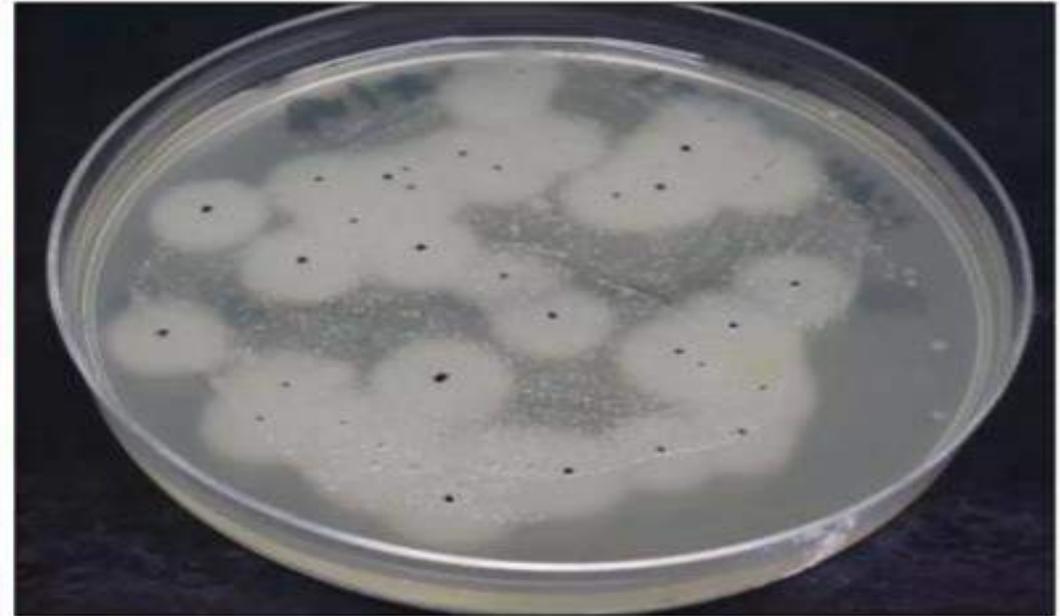
**Double zone of hemolysis** *Clostridium perfringens*

# *C. perfringens* (culture)

- Samples are cultured on **blood agar plates (BAP)** anaerobically (i.e. using jar), **Tryptose sulphite cycloserine agar (TSCA)**, **Robertson's Cooked Meat broth** & **Thioglycolate broth media**.



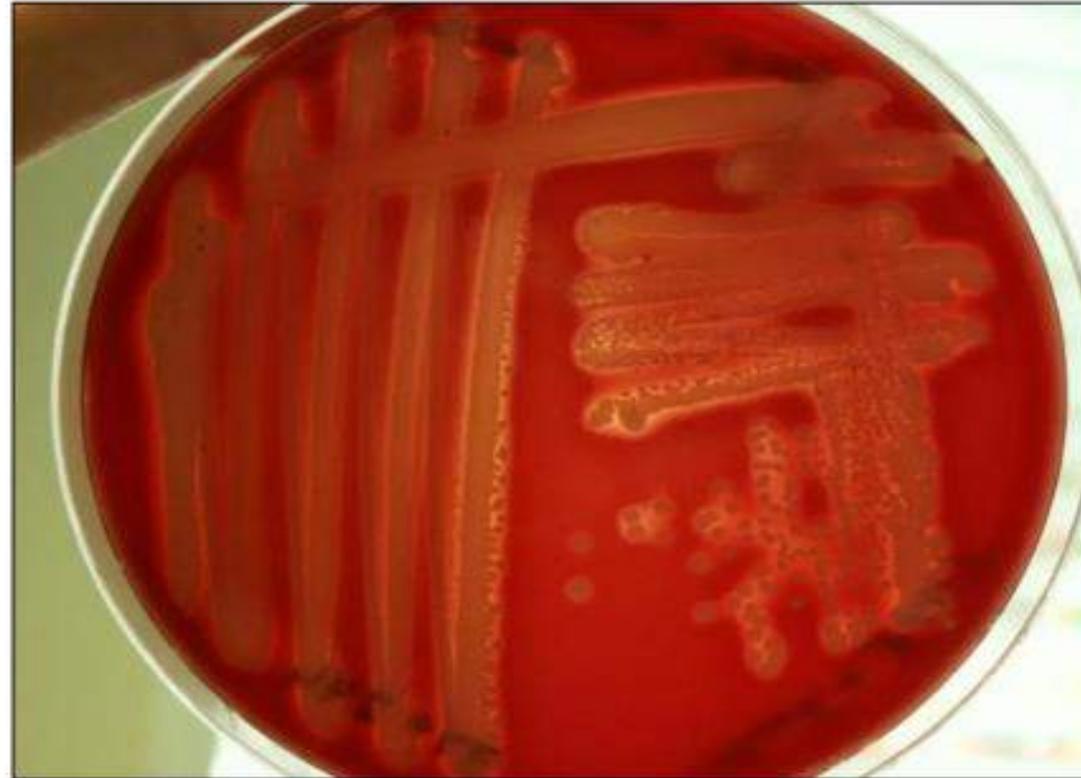
*Double zone of clear beta-hemolysis around a colony is clearly seen (BAP)*



*TSCA (*C. perfringens*)*

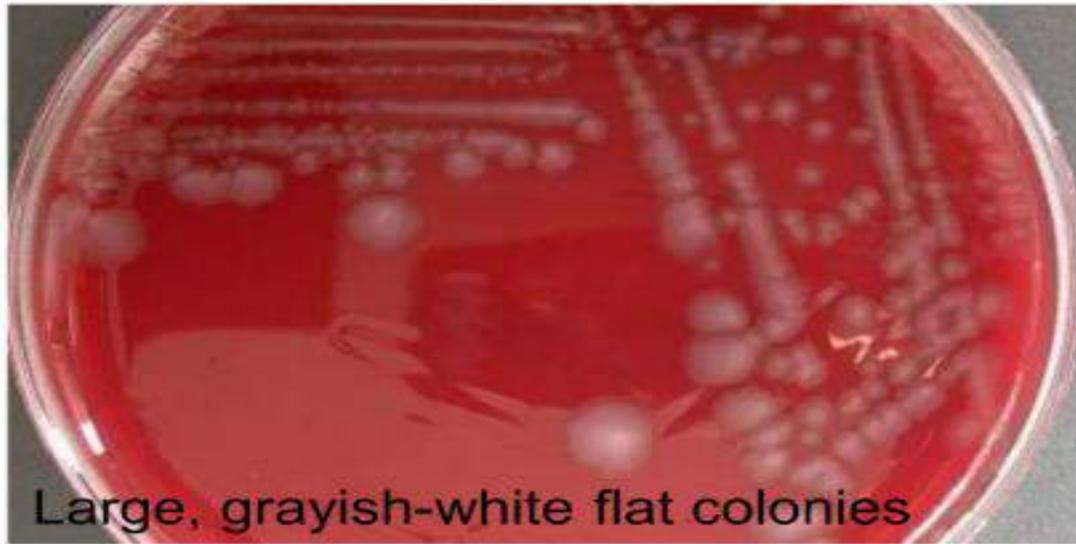
# *C. botulinum* (culture)

- On blood agar *C. botulinum* produces large semi-transparent colonies with a wavy outline.
- Most strains are beta-hemolytic.



# *C. difficile* (culture)

- **Blood agar (anaerobically)**: large, flat colonies with barnyard smell.
- **Cycloserine Cefoxitin Fructose Agar (CCFA)/ selective**: 4 mm colonies appear as yellow and ground glass-like and have filamentous edge, the odor associated with colonies is very distinct and typically like elephant or horse manure.



Large, grayish-white flat colonies

*Blood agar*



*CCFA*

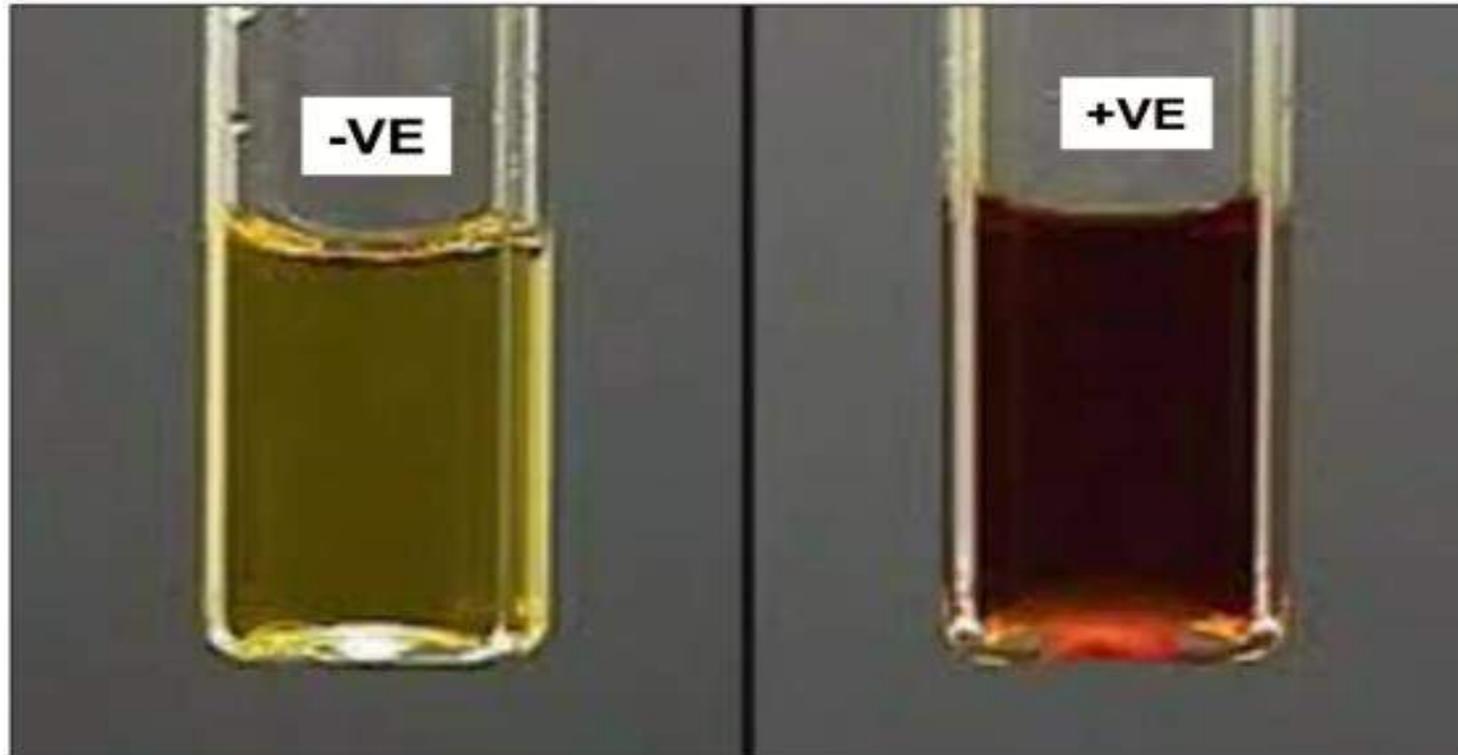
## ***C. tetani* (culture)**

- On blood agar it produces a fine film of growth.
- Swarming due to its motility.
- On fresh BAP it is hemolytic (alpha first followed by beta hemolysis).



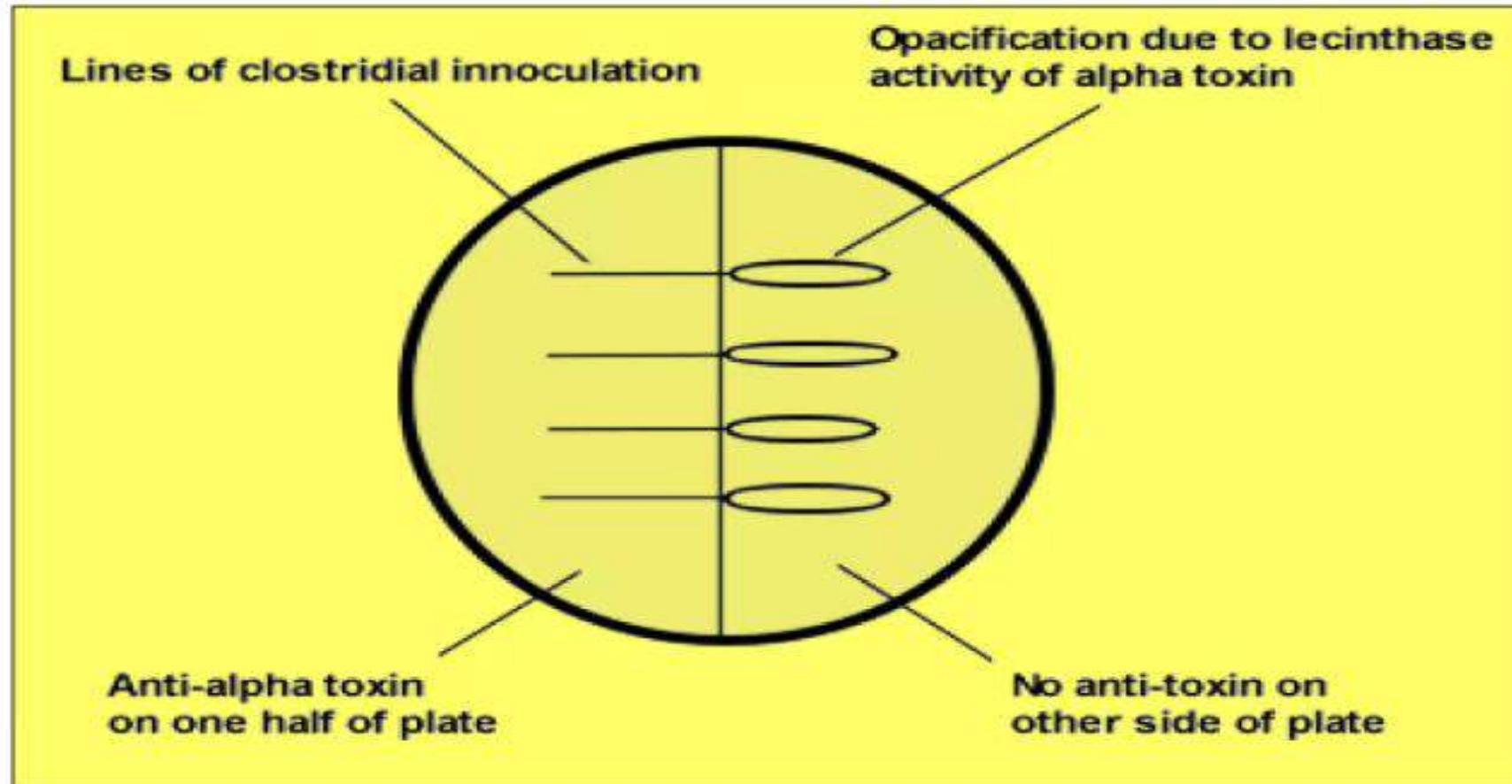
# BIOCHEMICAL TESTS

- **Nitrate reduction test:** *C. perfringens* can reduce nitrate to nitrite which is indicated by **red** colour after addition of reagents.

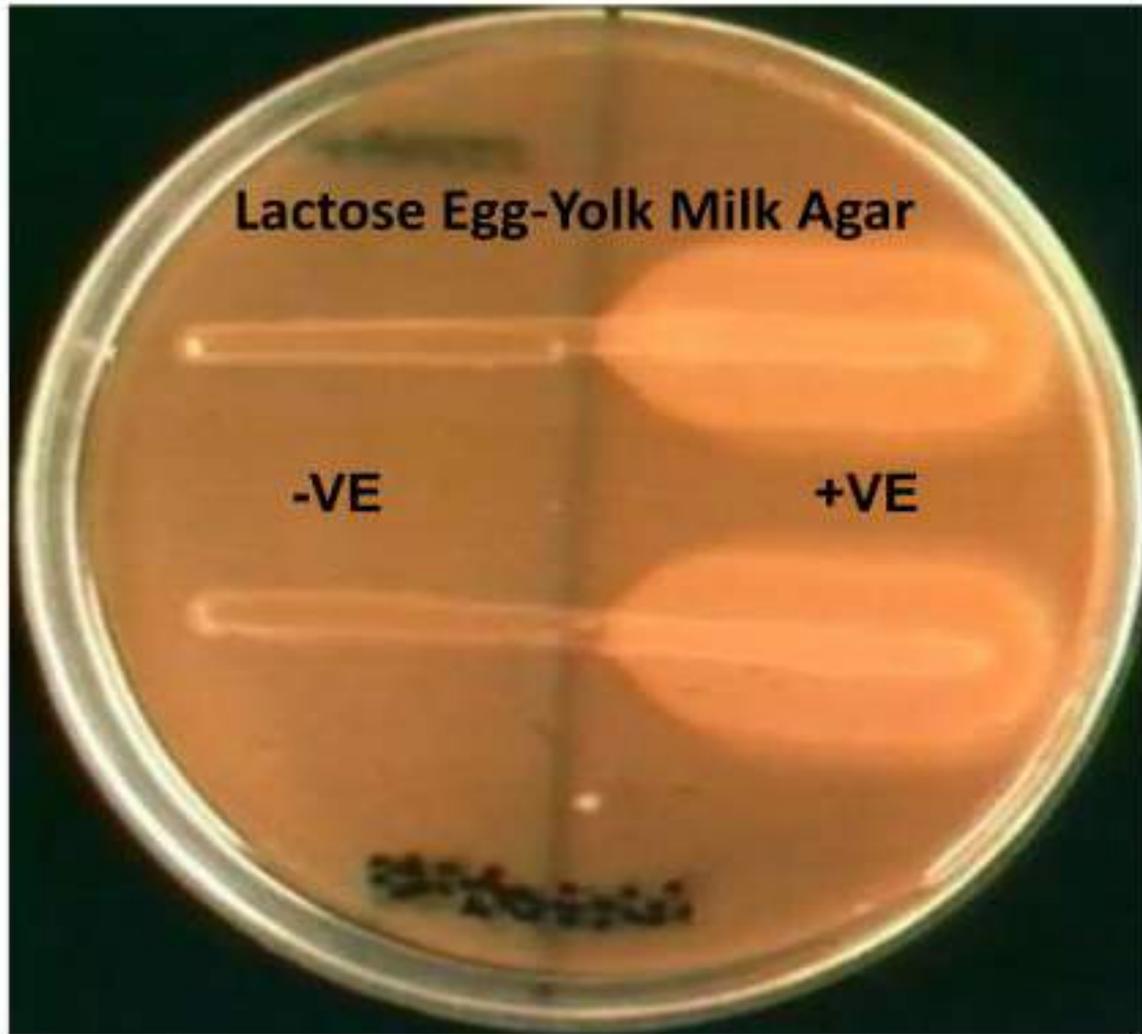


# BIOCHEMICAL TESTS

- **Nagler reaction:** *C. perfringens* is Nagler positive due to lecithinase activity of alpha exotoxin.



# NAGLER TEST



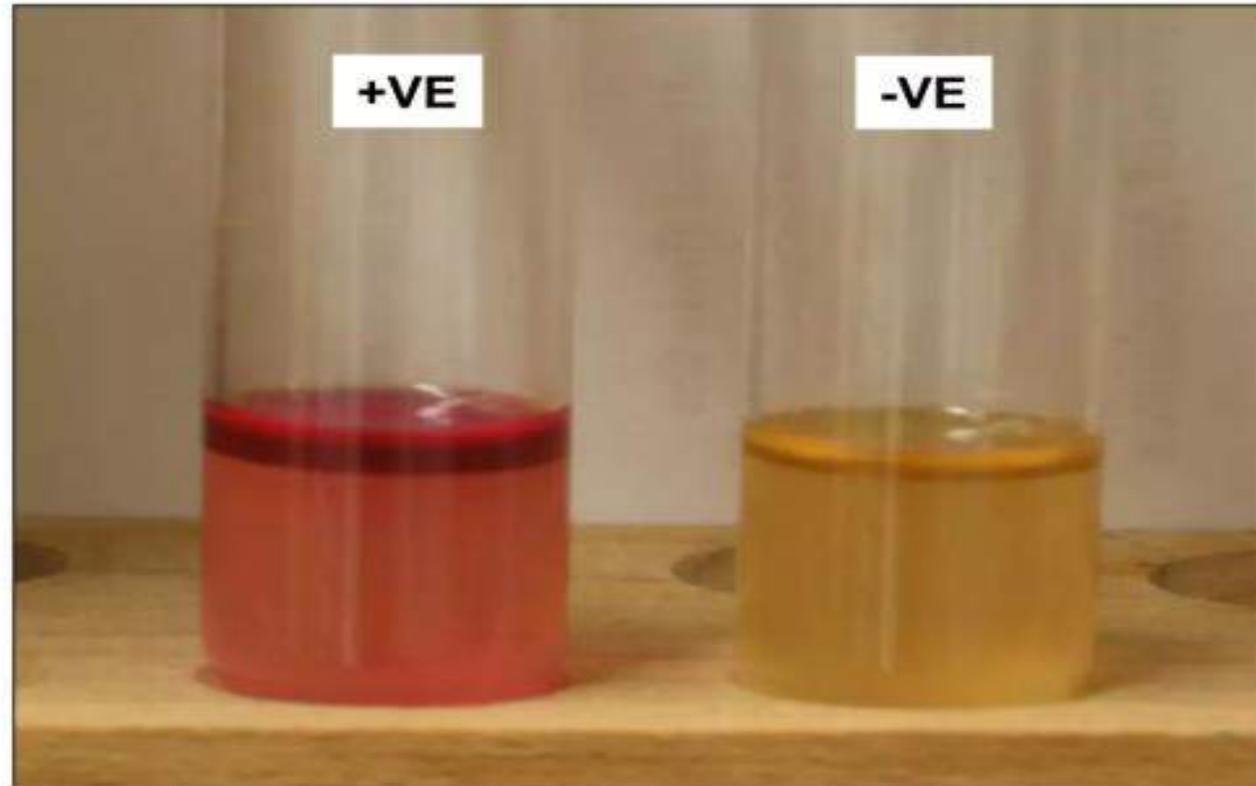
## **Lecithinase activity :**

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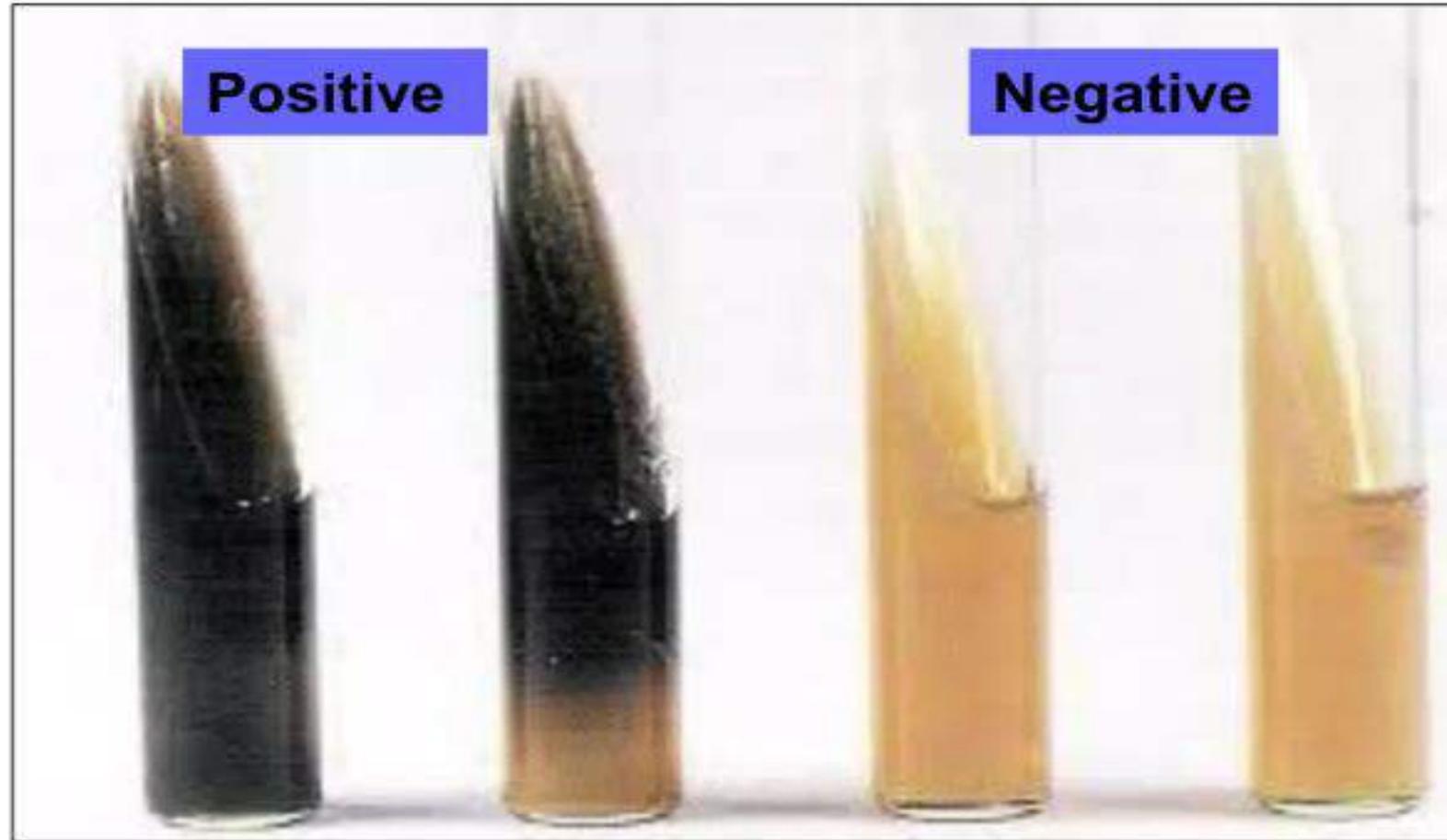
**is the ability of an enzyme called lecithinase to break down lecithin, a type of phospholipid found in cell membranes. This breakdown produces an insoluble product, diacylglycerol, which forms a visible white, opaque zone of precipitation around the colony when bacteria are grown on a medium like egg yolk agar. This activity is used to identify bacteria, as many pathogenic and foodborne microbes, like *Clostridium perfringens* and *Staphylococcus aureus*, produce lecithinase.**

# BIOCHEMICAL TESTS

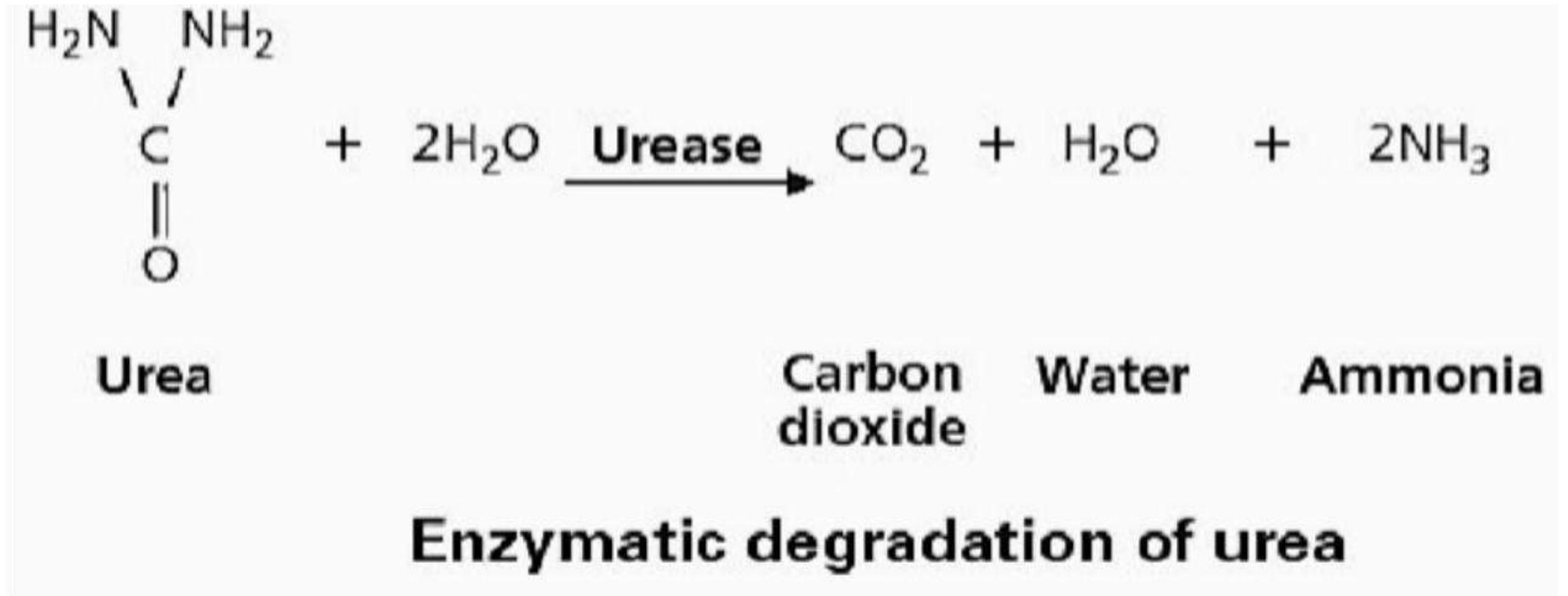
- **Indole test:** *C. tetani* is cultured in tryptophan broth and Indole production is detected by the addition of Kovac's reagent. A positive indole reaction is indicated by formation of red colour ring.



- **Bile esculin test:** *C. difficile* grows in bile esculin agar and hydrolyze esculin which is indicated by production of black colour.



- The **urease test** determines whether a microorganism can hydrolyze urea, releasing a sufficient amount of ammonia to produce a color change by a pH indicator. Urease hydrolyzes urea to form ammonia, water, and CO<sub>2</sub>.

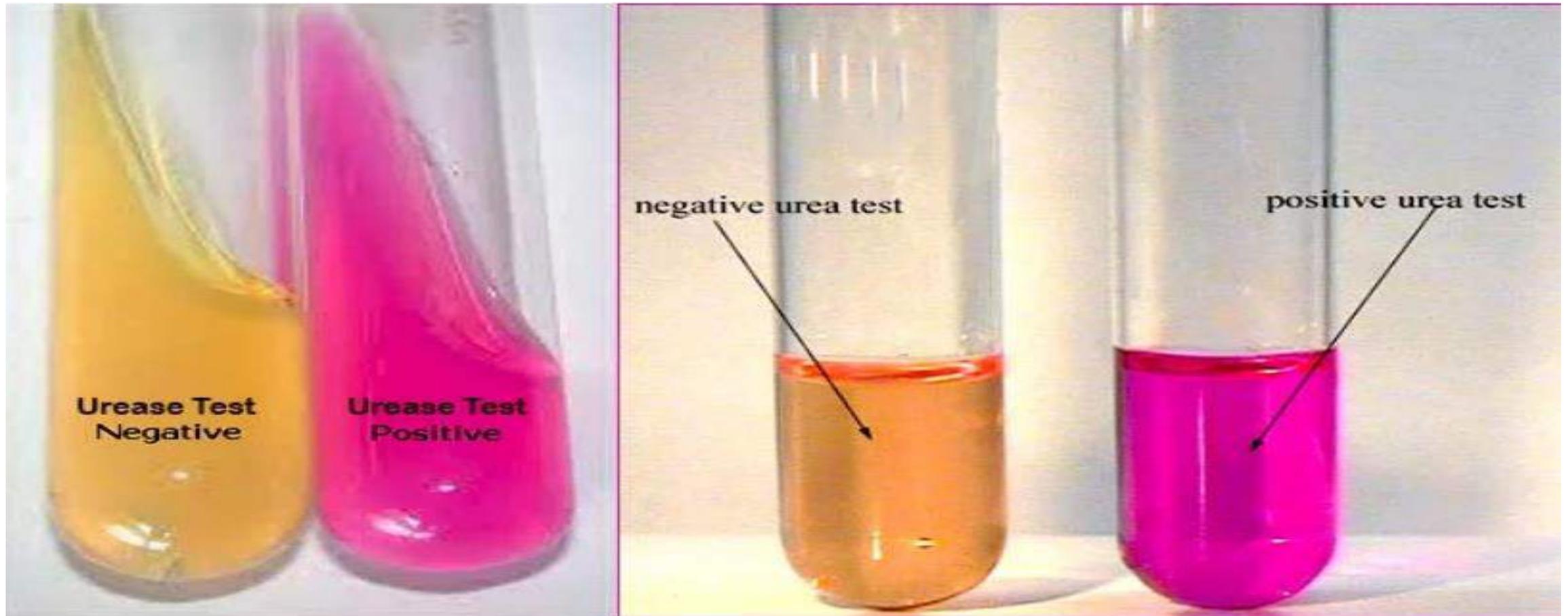


- **Urease Test**

The **urease test** determines whether a microorganism can hydrolyze urea, releasing a sufficient amount of ammonia to produce a color change by a pH indicator. Urease hydrolyzes urea to form ammonia, water, and CO<sub>2</sub>.

- Different formulations of urea agar are available, but **Christensen's urea agar** is generally preferred. The surface of the agar slant is inoculated but not stabbed. The resulting alkaline pH from hydrolysis of urea is indicated by a bright pink color.
- Many enteric bacteria (and a few others) possess the ability to metabolize urea, but only members of *Proteus*, *Morganella*, and *Providencia* are considered rapid urease-positive organisms.
- **Christensen's urea agar was formulated to differentiate rapid urease positive organisms from slower urease-positive and urease negative bacteria.**

Different formulations of urea agar are available, but **Christensen's urea agar** is generally preferred. The surface of the agar slant is inoculated but not stabbed. The resulting alkaline pH from hydrolysis of urea is indicated by a bright pink color. Many enteric bacteria (and a few others) possess the ability to metabolize urea, but only members of *Proteus*, *Morganella*, and *Providencia* are considered rapid urease-positive organisms.





## ⚡ Key Biochemical Characteristics of Pathogenic Species

| Species               | Gram Reaction | Motility                 | Hemolysis                      | Gas Production | Lecithinase (Egg yolk agar)      | Lipase               |
|-----------------------|---------------|--------------------------|--------------------------------|----------------|----------------------------------|----------------------|
| <i>C. botulinum</i>   | +             | Motile                   | $\beta$ (some strains)         | +              | -                                | + (iridescent sheen) |
| <i>C. tetani</i>      | +             | Motile (swarming growth) | -                              | +              | -                                | -                    |
| <i>C. perfringens</i> | +             | Non-motile               | Double zone $\beta$ -hemolysis | +++            | + (opaque zone on egg yolk agar) | -                    |
| <i>C. difficile</i>   | +             | Motile                   | -                              | +              | -                                | -                    |