

Lab.13. gram positive rods (*Clostridium* spp.)**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.
- pathogens are *C. tetani* (tetanus), *C. botulinum* (botulism), *C. perfringens* (food poisoning and gas gangrene).
- 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

Spores

- Spores of *C.botulinum*: survive boiling after 3-4 hrs., even at 105°C are not killed completely.
- Spores of *Cl.perfringens* are destroyed by boiling in 5 minutes.
- Spores of *Cl.tetani* persist for years in dry soil, while few strains resist boiling for 15-90 min.
- All species are killed by autoclaving at 121°C for 20 minutes.
- Halogens are effective; 1% aq. Soln . of Iodine kills spores within 3 hrs. 2% glutaraldehyde kills spores

Specimens

C. perfringens: wound swabs, necrotic tissues, muscle fragments, pus, stool and food (food poisoning)

C. botulinum: food, faeces or intestinal contents Diabetic foot

C. tetani : Excised tissue bits from the necrotic depths of wounds are more reliable than wound swabs.



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.



Cultures

C. perfringens

Samples are cultured on **blood agar plates** anaerobically (i.e. using jar). Double zone of clear α and β -hemolysis around a colony is clearly seen .



C. botulinum

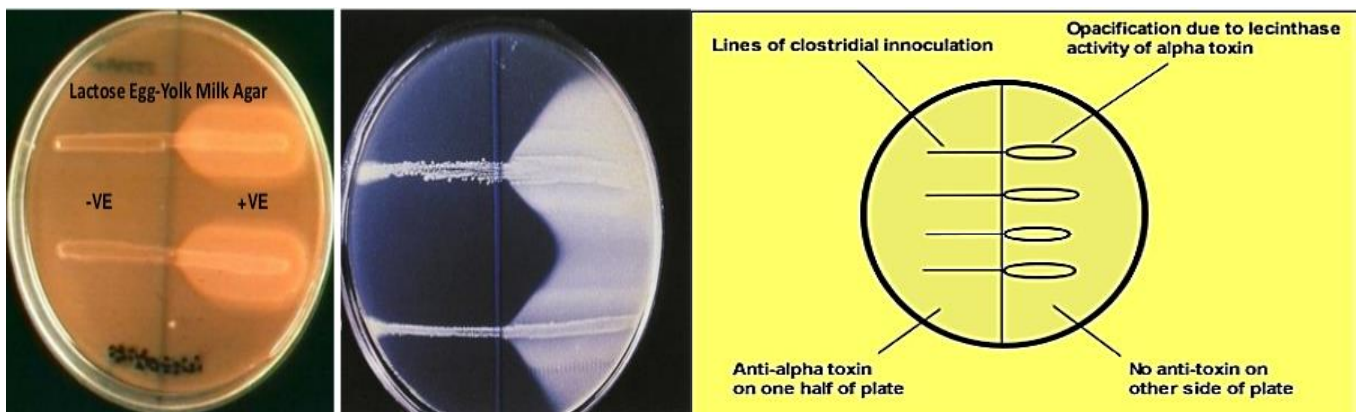
On blood agar *C. botulinum* produces large semi-transparent colonies with a wavy outline. Most strains are **β -hemolytic**.

***C. tetani***

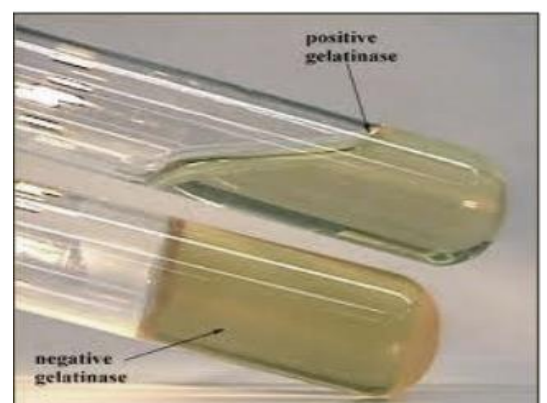
On blood agar it produces a fine film of growth. Swarming due to its motility.

**BIOCHEMICAL TESTS****Nagler reaction:**

C. perfringens is Nagler positive due to lecithinase activity of alpha exotoxin.

**BIOCHEMICAL TESTS****1. Gelatin liquefaction test:**

C. perfringens produces proteolytic enzyme (gelatinase) that liquefy gelatin.



2. 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.

