MICROBIOLOGY LAB . 7

Gram- positive rods

Spore formers

Bacillus & Clostridium

lect.
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Learning Objectives:

After this lab. You must be able to:

- Distinguish between G +ve rods genera.
- Describe each species of Gram positive rods microscopically and culturally.
- Differentiate between *Bacillus anthracis* and other saprophytic species.
- Differentiate between *Clostridium* spp.
- List types of clinical infections these organisms produce
- Predict G +ve causative agents causing clinical cases.
- Discuss the principles of identifying tests.
- Know prevention ways of some organisms.

CLINICALLY IMPORTANT GRAM POSITIVE RODS

There are four medically important genera are:

A. Spore forming

1- Bacillus

2- Clostridium

longer and more deeply staining than others.

B. Non spore forming

1- Corynebacterium (club-shaped)

2- Listeria

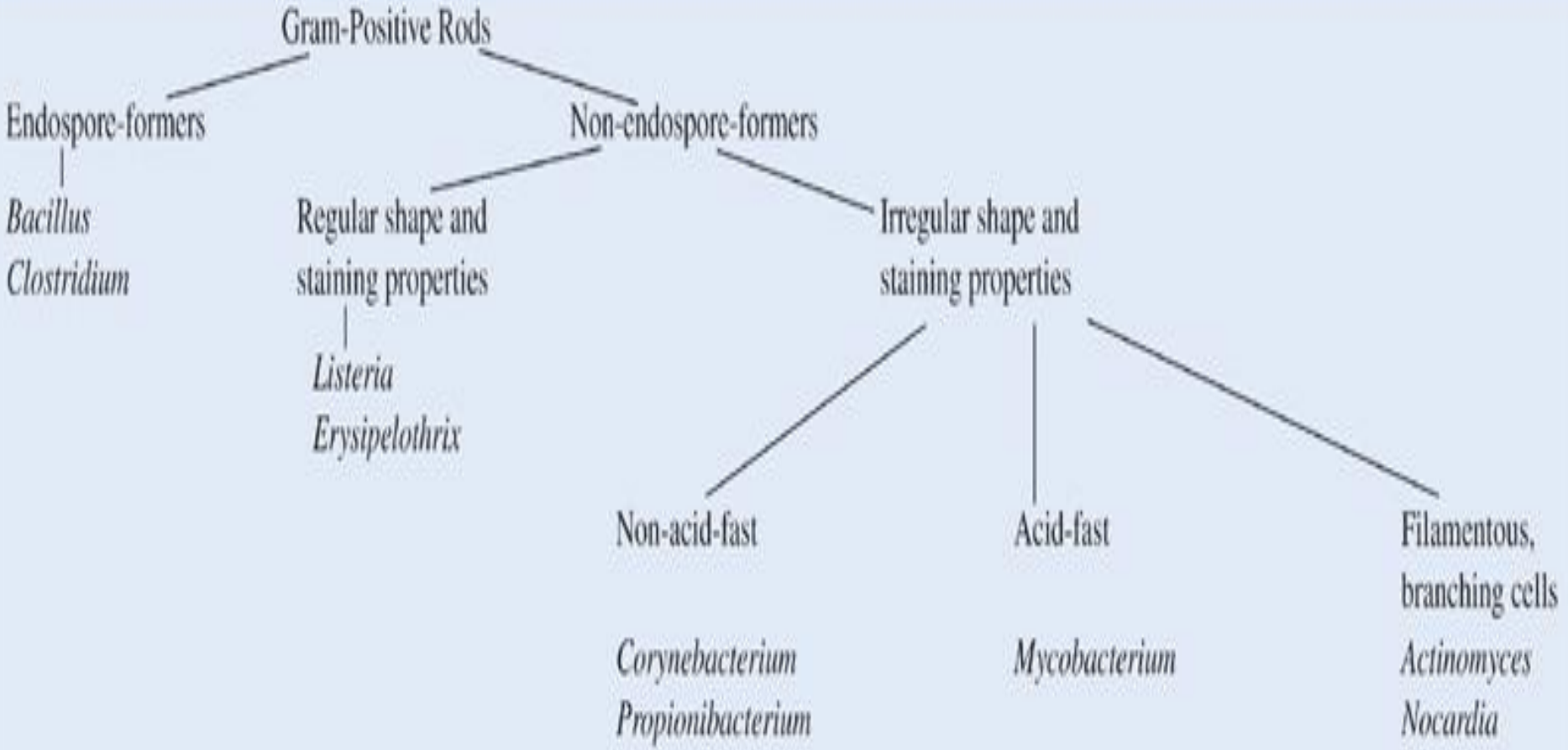
(Chinese letters).

Aerobic Spore-forming Gram-positive Rods

(Bacillus)

TABLE 19.1

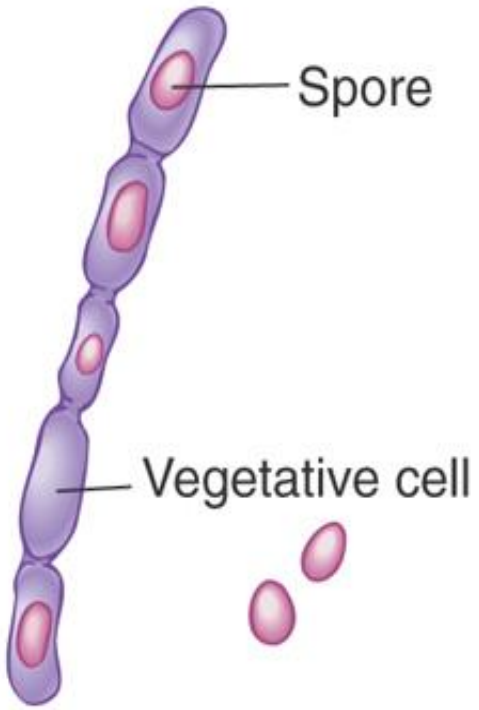
Gram-Positive Bacilli



Bacillus

General Characteristics of Bacillus:

- Large gram-positive rods have square ends.
- They are frequently arranged in long chains.
- **Aerobic,** produce endospores located in the center of the bacilli.
- Most members are saprophytic organisms , primary habitat in soil.
- **Catalase positive** (most)
- Can flourish at extremes of acidity & alkalinity (pH 2 to 10)



(a)

Diseases Associated with Bacillus

Organism	Diseases
*B. anthracis**	Anthrax (cutaneous, gastrointestinal, inhalation)
*B. cereus**	Gastroenteritis (emetic, diarrheal), ocular infections, catheter-related sepsis, opportunistic infections
*B. mycoides**	Gastroenteritis, opportunistic infections
*B. thuringiensis**	Gastroenteritis, opportunistic infections
Other *Bacillus* species	Opportunistic infections

Bacillus anthracis

1. Bacillus anthracis: causative agent of anthrax

1. Large, block-shaped or bamboo- shaped rods.
2. Produce central spores that develop under all conditions except in the living body
3. Virulence factors – polypeptide capsule and exotoxins
4. There are 3 types of anthrax:
   - **Cutaneous** (malignant pustule)– spores enter through skin, black sore- eschar; least dangerous
   - **Pulmonary** (wool sorter's disease)–inhalation of spores, most deadly form.
   - **Gastrointestinal** – ingested spores, rare but commonly fatal disease.

Cutaneous anthrax

About 20% mortality

Lab Diagnosis:

- **Specimen:** aspirate or swab from cutaneous lesion, blood
- **Gram stain:** large, square-ended gram-positive rods; may appear end-to end giving a "bamboo appearance“.
- **Culture:** Nonhemolytic on blood agar; raised, large, grayish-white, irregular, fingerlike edges described as “Medusa head” or “beaten egg whites’’ with ground-glass appearance.
  comma shape outgrowth may project from the colony, “string pearl reaction”

Key Characteristics to Distinguish between B. anthracis & Other Species of Bacillus

Characteristic	*Bacillus anthracis*	Other *Bacillus* spp.
Capsule	Pos	Neg
Hemolysis	Neg	Pos
Motility	Neg	Pos (usually)
Gelatin hydrolysis	Neg	Pos
Medusa head	Pos	Neg

Bacillus cereus

2- *Bacillus cereus*: causes food poisoning

- Large, motile (swarming), saprophytic bacillus
- Produce two toxins:
  - ✓ heat and acid stable toxin (Emetic syndrome) Associated with fried rice
  - ✓ Heat labile enterotoxin (Diarrhoeal disease) Associated with meat, poultry, and soups
- Lab diagnosis – Demonstration of large number of bacilli in food. And it not usually done.

Bacillus cereus

Foodborne Diseases of B. cereus

(Intoxication) (Foodborne Infection)

	Emetic Form	Diarrheal Form
Implicated food	Rice	Meat, vegetables
Incubation period (hours)	<6 (mean, 2)	>6 (mean, 9)
Symptoms	Vomiting, nausea, abdominal cramps	Diarrhea, nausea, abdominal cramps
Duration (hours)	8–10 (mean, 9)	20–36 (mean, 24)
Enterotoxin	Heat-stable	Heat-labile

Anaerobic Spore-forming Gram-positive Bacilli

(Clostridia)

General features:

All clostridia are:

- Gram positive, straight or slightly curved rods with slightly rounded ends.
- Anaerobes (acquire energy only by fermentation and grow well on the blood agar and other media).
- Spore forming (Spores of clostridia are usually wider than the diameter of the rods).

Clostridia:

There are four medically important species; *Cl. tetani, Cl. botulinum, Cl. perfringens, and Cl. Difficile*

Species	Human Disease	Frequency
C. difficile	Antibiotic-associated diarrhea, pseudomembranous colitis	Common
C. perfringens	Soft tissue infections (i.e., cellulitis, suppurative myositis, myonecrosis or gas gangrene), food poisoning, enteritis necroticans, septicemia	Common
C. septicum	Gas gangrene, septicemia	Uncommon
C. tertium	Opportunistic infections	Uncommon
C. botulinum	Botulism	Uncommon
C. tetani	Tetanus	Uncommon
C. barati	Botulism	Rare
C. butyricum	Botulism	Rare
C. histolyticum	Gas gangrene	Rare
C. novyi	Gas gangrene	Rare
C. sordellii	Gas gangrene	Rare

Clostridium tetani

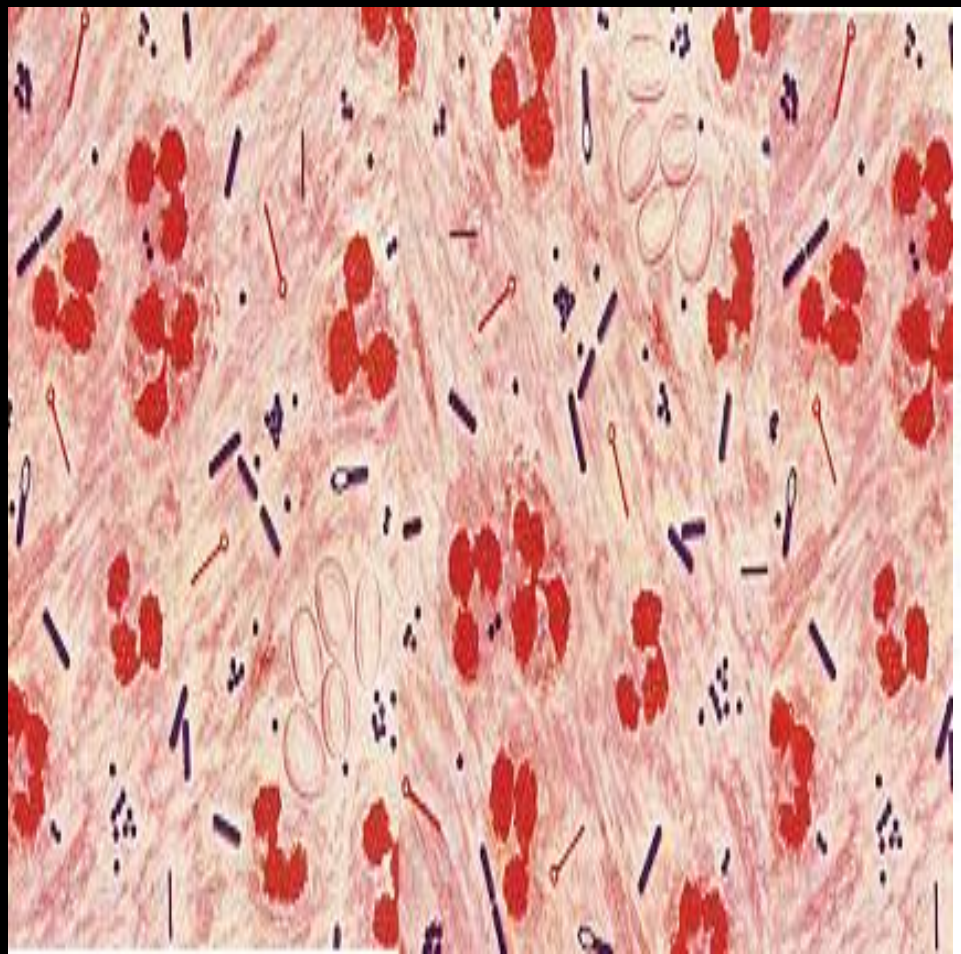
Cl. tetani:

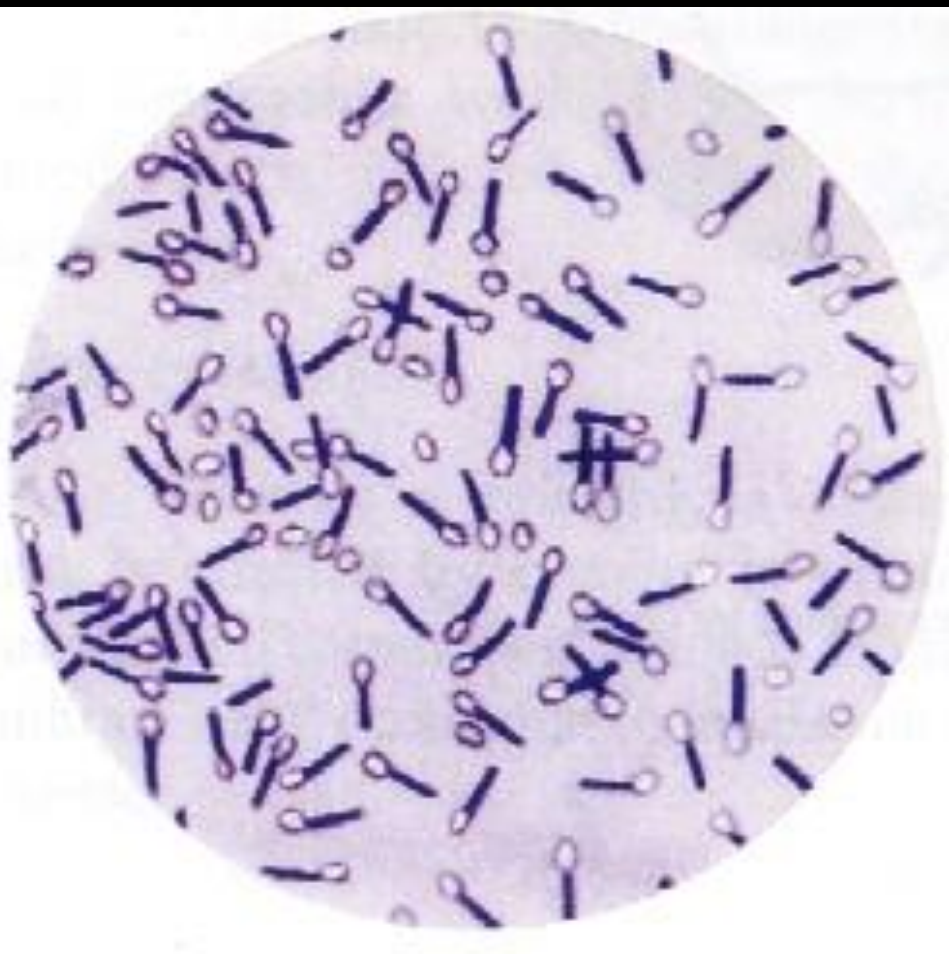
The causative agent of tetanus (lockjaw).

Important features:

- Form rounded or oval , terminal spores (2-4 times the diameter of bacillus, (drumstick appearance)
- Motile with peritrichous flagella and produces a thin spreading film (swarming) when grown on enriched blood agar
- Grows well in cooked meat broth
- Produce very powerful exotoxins (tetanolysin- causes lysis of RBCs, tetanospasmin- neorotoxin)
- Proteolytic does not ferment sugars

CLOSTRIDIUM TITANI





Clinical findings:

- Strong muscle spasm (**spastic paralysis**).
- Specific clinical features:
  - Lockjaw (trismus)
  - Risus sardonicus
  - Opisthotonos.

trismus, risus sardonicus, opisthotonos

Prevention: toxoid, 3% formaldehyde

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
- **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
- **Culture:** anaerobically
  - **On BA:** growth appears as a fine spreading film (swarming).The bacilli may produce hemolysis (alpha to beta) due to tetanolysin
  - **On NA:** after 24 – 72 hrs incubation are irregularly round, glistening grayish- yellow translucent colonies and the edge is filamentous
  - **On RCM:** produce some gas and blackened meat.
  - **Gelatin medium:** fir tree type of growth.

Treatment :

- Immune globulin to neutralize the toxin.
- Penicillin G or metronidazole to kill the toxin-producing bacteria.

Prevention:

- Immunization with tetanus toxoid (formaldehyde-treated toxin) which is usually given in combination with Diphtheria and Pertusis vaccines (DTaP)

Clostridium botulinum

Cl. botulinum:

The causative agent of botulism

Important features:

- Form oval, bulging sub-terminal spores wider than the bacilli.
- Motile
- Producing the most powerful exotoxin .
- Result in flaccid paralysis can lead to respiratory failure.
- Associated with canned food , the highest food are:

1- alkaline vegetables

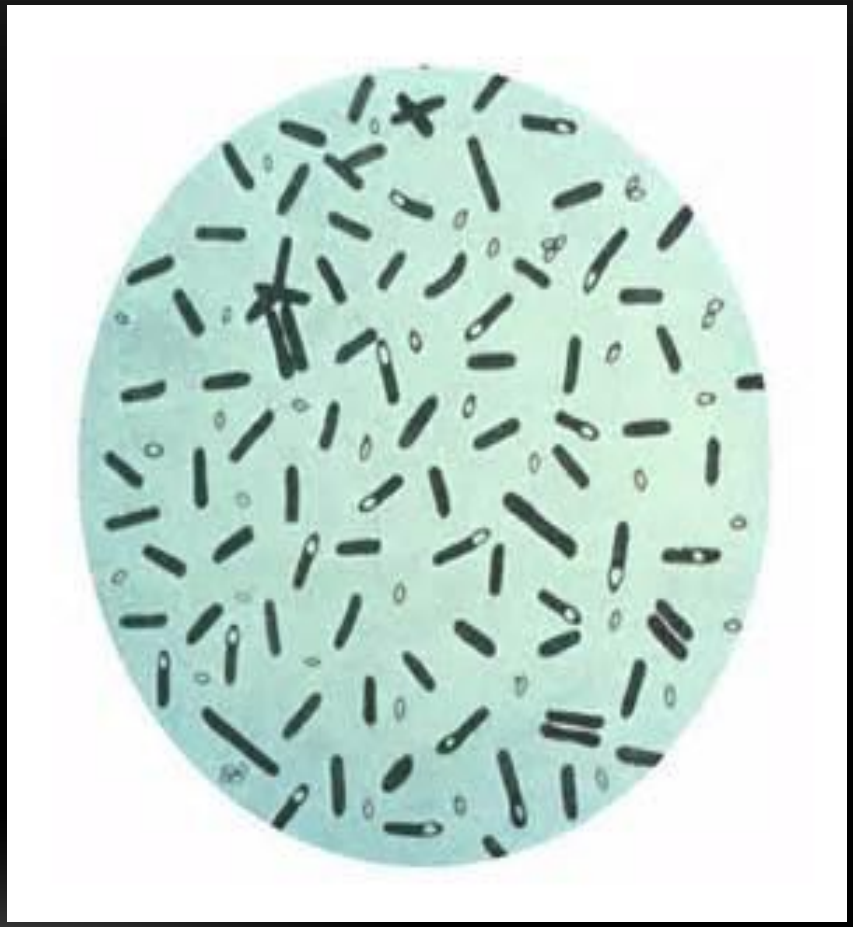
2- smoked fish.

Laboratory Diagnosis Of Botulism

Botulinum confirmed by isolating the organism or detecting the toxin in food products or the patient feces or serum.

* microscopic detection or culture are often unsuccessful (few organism and slow growing)

** toxin detected and typed in lab via toxicity and antitoxin neutralization test in mice or ELISA.



TREATMENT AND PREVENTION

- Prompt antitoxin can be life-saving
  (mortality 100%→25%)
- Airway protection and respiratory support
- There is no vaccine
- Prevention relies on regulated food manufacturing

Clostridia perfringens

Cl. Perfringens: the causative agent of gas gangrene (myonecrosis) and food poisoning

Important features:

1. Form oval, not bulging, central or sub-terminal spores.
2. Capsulated in animal tissues.
3. Non motile, but rapid spreading growth mimics motile organism.
4. Produce variety of toxins, but the most important is alpha toxin (lecithinase) responsible for histotoxic and enterotoxigenic infection in humans.
5. Produce cellulitis in the wound area, Crepitation indicates gas in tissue
6. Produce two zone of hemolysis around the colonies, first zone is due to theta toxin and the second is due to alpha toxin

LABORATORY DIAGNOSIS

- **Specimen:** Histological specimen or wound exudates, food and feces
- **Microscopical examination:** Large gram-positive bacilli, spores are rarely observed .
- **Culture:** Anaerobically at 37ºC
  - **On Robertson's cooked meat medium** → blackening of meat will observed with the production of H_2S and NH_3
  - **On blood agar** double zone of hemolytic colonies.
  - Thioglycollate broth (contains sodium thioglycollate)
- Biochemical test:
  - It ferments many carbohydrates with acid & gas
  - It acidified litmus milk with stormy clot production
  - Nagler's reaction is positive

LITMUS MILK REACTION:

- **Principle:** milk contain lactose with three main proteins; casein, lactoalbomin and lactoglobuline therefore, an organism may exhibit one or more of the following metabolic properties:

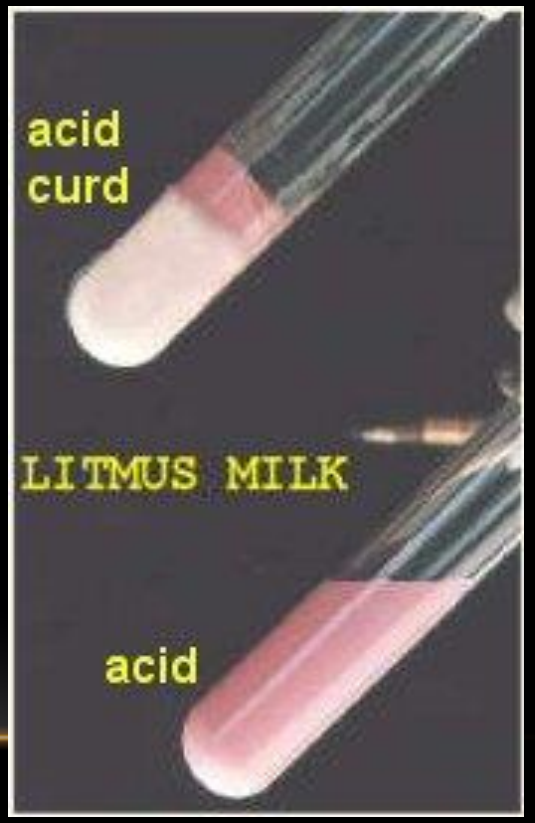
- Lactose fermentation
- Litmus reduction
- Clot formation
- Peptonization (digestion)
- Gas formation

REACTION ON LITMUS MILK

1- Acidic Reaction

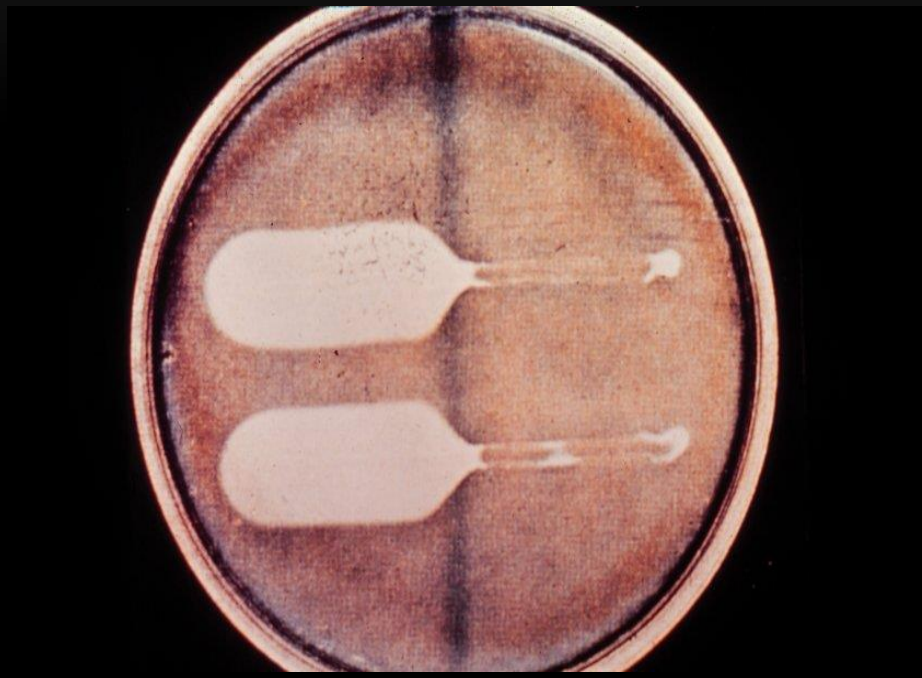
2- Basic Reaction

REACTION ON LITMUS MILK

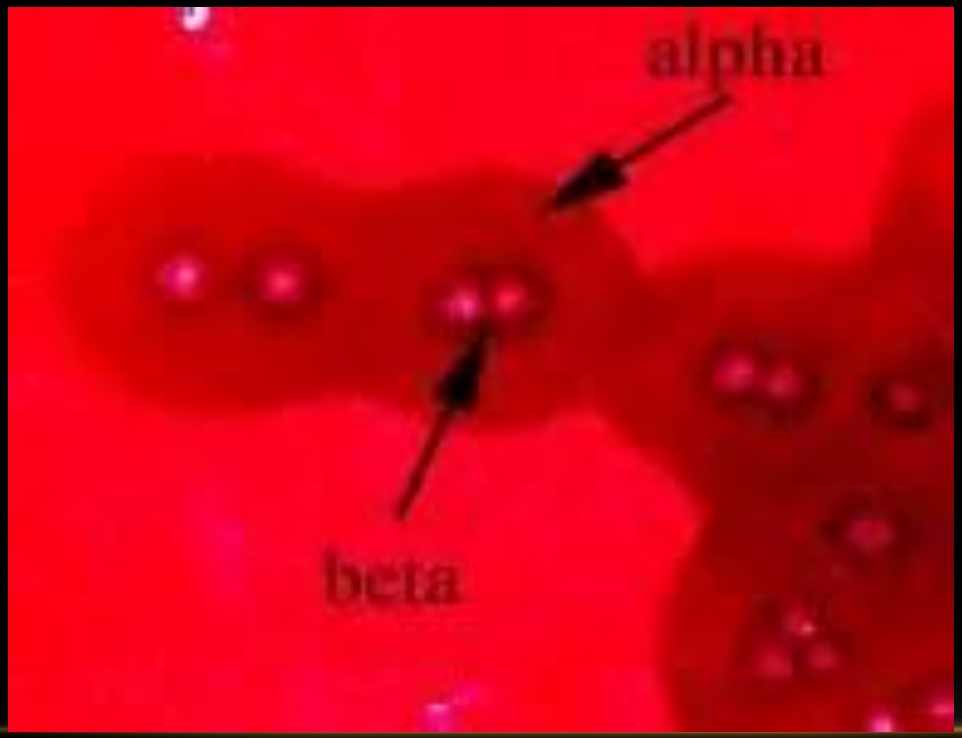


Nagler's Reaction:

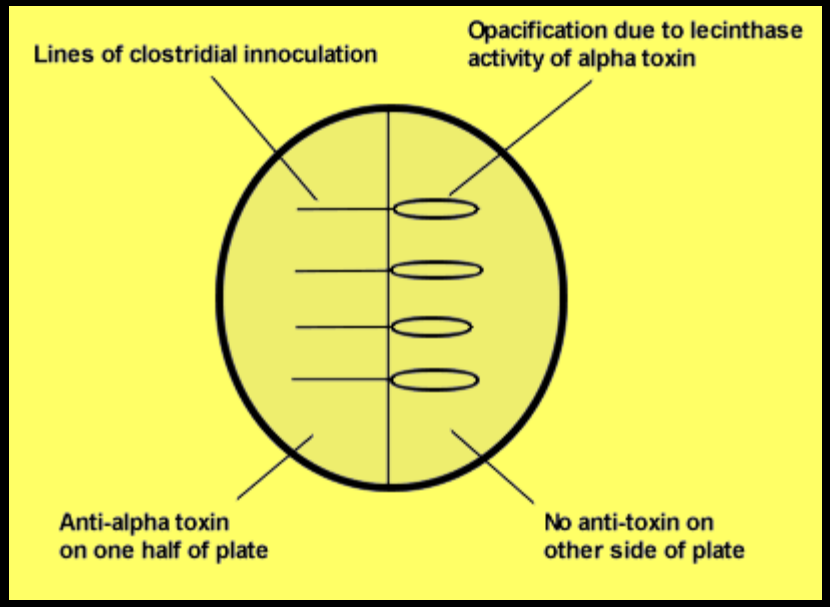
- Presumptive identification of *Cl. perfringens*
- alpha toxin (lecithinase) breakdown phospholipids (lecithin) in egg yolk agar to insoluble diglycerides resulting in an opaque halo (becomes turbid)
- This activity is specifically blocked by antitoxin



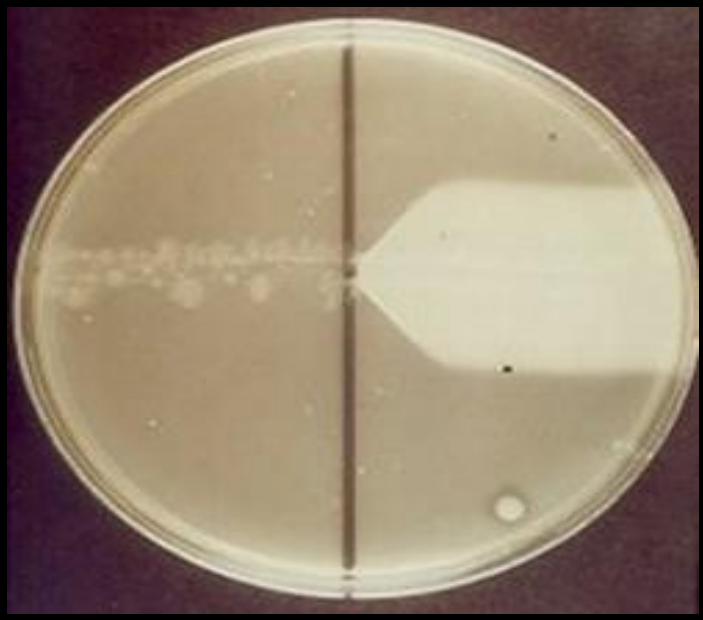
hemolysis on blood agar



NAGLER REACTION

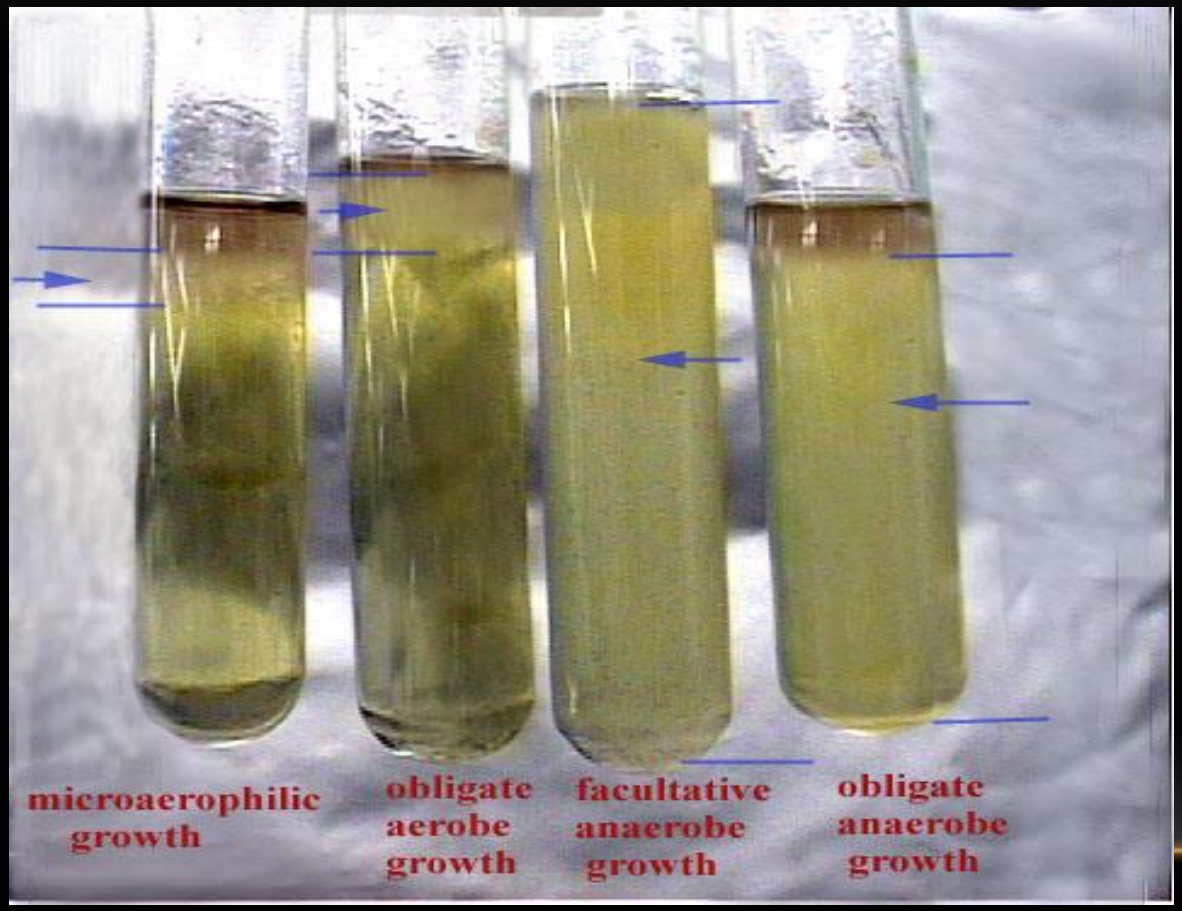


Procedure of Nagler Reaction



Positive Nagler Reaction

GROWTH ON FLUID THIOGLYCOLATE



Clostridium sporogenes Growing in Thioglycolate Medium

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

TREATMENT:

- **Early diagnosis and aggressive treatment essential**
- **Removal of necrotic tissue (surgical debridement)**
- **Penicillin G in high doses if more serious infection**

Clostridia difficile

Cl. Difficile: the causative agent of antibiotic associated pseudomembranous colitis.

- Important features:

- GIT normal flora in 3-30% of population

- Extremely oxygen sensitive

- Laboratory diagnosis:

- Isolating the organism or detecting the cytotoxin or enterotoxin in pateint's feces by ELISA.