CHAPTER III
GRAM POSITIVE RODS

Learning objective:

At the end of this chapter the students will be able to:

- List the medically-important species of Gram positive rods
- Describe general characteristics of Gram positive rods
- Recognize diseases caused by Gram positive rods
- Describe the virulent factor of pathogenic species of Gram positive rods
- Discuss pathogenicity, clinical manifestations, laboratory diagnosis, prevention & control of members of the Gram positive rods

Genus Bacillus:

General characteristics

- Large gram positive (young/fresh culture) bacilli with square end.
  - Some are gram negative in old culture
- Aerobic or facultative anaerobe
- Spore former
- Include thermophilic, psychrophilic, acidophilic, alkalophilic, halotolerant, halophilic
- Majority are harmless, saprophyticus
- Some opportunistic or obligate pathogens of animals are present
- At least 48 species are known but only *B. anthracis* and *B. cereus* cause disease in humans.

Natural habitat

- Saprophytes, widely distributed in natural environment (mainly in soil and water)
- Contaminants of operating rooms and surgical dressings, pharmaceutical products and dried foods

B. anthracis

- *B. anthracis* produces a single antigenic type of capsule and several exotoxins.
- *B. anthracis* is responsible for the disease anthrax.

B. anthracis…Cont'd

Virulence factors

- Capsule, plasmid encoded
- Anthrax toxin (AB toxin)
  - consists three distinct components/Thermostable proteins
    - Edema factor (EF)
    - Lethal factor (LF)
    - Protective antigen (PA)

B. anthracis…Cont'd

Pathogenesis

- *B. anthracis* causes anthrax which is mainly a disease of sheep, cattle, goats and other herbivores with humans becoming infected only after coming into contact with infected animals or their skins.
- Human infections (zoonoses) can occur from handling infected animals or coming into contact with skins containing anthrax spores, e.g. when using skins as clothing, water-carrying containers, or sleeping mats.
- Other sources of infection include animal hair, bones, and the bedding of infected animals.
- Less commonly, infection is caused by eating infected meat.

B. anthracis…Cont'd

Depending on the source and site of infection, *B. anthracis* can cause:

***Cutaneous anthrax* (commonest form):**

- Bacilli enter damaged skin, producing a blister ('malignant pustule') which usually ulcerates and eventually forms a dry black scab surrounded by oedema.
- Fatal septicaemia, toxaemia, and meningoencephalitis may develop, especially in non-immune persons. Ocular anthrax may also occur.

***Pulmonary anthrax*:**

- Caused by inhaling large numbers of *B. anthracis* spores ('woolsorters' disease).
- Infections are usually fatal.

B. anthracis…Cont'd

***Enteric anthrax*:**

- A severe form of gastroenteritis with fever, abdominal pain and bloody diarrhoea, due to ingesting infected meat.
- Septicaemia often develops.
- *Meningoencephalitis*: Usually as a complication of septicaemia and occasionally as primary anthrax meningoencephalitis.

Clinical findings

- Skin infection (malignant pustule)
- Mediastintis, sepsis, meningitis
- Hemorrhagic pulmonary edema
- Hemorrhagic pneumonia

Laboratory Diagnosis

Specimen: fluid or pus from a local lesion, blood, and sputum.

Microscopic

- Stained smears from the local lesion or of blood from dead animals often show chains of large gram-positive rods.
- **Loeffler's polychrome (McFadyean) methylene blue stained smear**
  - Square ended blue-black bacilli surrounded by a pink/purple capsule
- Anthrax can be identified in dried smears by immunofluorescence staining techniques.

Culture

- When grown on blood agar plates, the organisms produce non-hemolytic gray to white mucoid colonies with a rough texture and a ground-glass appearance.
- Comma-shaped outgrowths (Medusa head appearance) may project from the colony.
- Gram stained smear from culture shows large gram-positive rods.
- Grow on nutrient medium under aerobic/anaerobic conditions

Biochemical test:

- Ferment glucose, maltose, sucrose, trehalose, and dextrine, with acid production but not gas
- Positive for Nitrate reduction test, Catalase test, starch hydrolysis, VP, and gelatine liquefaction

- In semisolid medium, anthrax bacilli are always nonmotile, whereas related nonpathogenic organisms (eg, *B cereus*) exhibit motility by "swarming."
- Demonstration of capsule requires growth on bicarbonate-containing medium in 5–7% carbon dioxide.
- Lysis by a specific anthrax -bacteriophage may be helpful in identifying the organism.

Serological test

- **ELISA measure antibodies against edema and lethal toxins,**
- **Acute and convalescent sera obtained 4 weeks apart should be tested.**
- **A positive result is a fourfold change or a single titer of greater than 1:32.**

Animal pathogenicity test

- Virulent anthrax cultures kill mice or guinea pigs upon intraperitoneal injection.
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Prevention & control

- Disposal of animal carcasses by burning or by deep burial in lime pits
- Decontamination of animal products
- Wearing of protective clothes & gloves for handling infected materials
  - **Eg.** Animal's hair, hide bone
- Immunization of domestic animals with live attenuated vaccines
- Immunization of individuals with high occupational risk
  - **E.g.** Leather factory

Bacillus cereus

- *B. cereus* is predominantly responsible for food poisoning in humans
- *B cereus* produces toxins that cause disease that is more an intoxication than a food-borne infection.

Pathogenesis

- *B. cereus* produces enterotoxins that causes food poisoning
- It causes two distinct forms of food poisoning
  - the emetic type, associated with fried rice, and
  - the diarrheal type, associated with meat dishes and sauces.
- *B cereus* is an important cause of eye infections, severe

  keratitis, endophthalmitis, and panophthalmitis.
- Typically, the organisms are introduced into the eye by

  foreign bodies associated with trauma.
- *B cereus* has also been associated with localized infections and with systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections.

- Occasionally *B. cereus* causes opportunistic infections in immunocompromised persons,
  - e.g. pneumonia, bacteraemia, wound infections.

Clinical manifestation

- Food poisoning caused by B. cereus is manifested by nausea, vomiting, abdominal cramps, and occasionally diarrhea and is self-limiting, with recovery occurring within 24 hours.

Lab diagnosis

- Suspected food (eg, rice, meat, vegitable) and vomitus of patients are cultured on ordinary media.
- Smear from colonies show large gram positive sporing bacilli
- *B. cereus,* unlike *B. anthracis,* is motile, non-capsulate, and produces haemolytic colonies on blood agar.
- The organism is non-lactose fermenting, producing pale colonies on MacConkey agar.
- On egg-yolk agar, *B. cereus* gives a strong lecithinase reaction. It rapidly liquefies gelatin stabs.

- Mannitol egg-yolk phenol-red polymyxin agar (MYPA) is recommended as a selective medium for the isolation of *B. cereus* from faeces, vomit, or food.
- After overnight incubation at 35–37 ºC, large 3–7 mm flat, dry grey-white colonies surrounded by an area of white precipitate are produced.
- *B. cereus* produces *beta*-lactamase and is resistant to penicillin and cephalosporins. Antimicrobials with activity against
- *B. cereus* include gentamicin, erythromycin, vancomycin and clindamycin.

Prevention and control

- Rice should not be kept warm for a long period of time

Genus Clostridium

General characteristics

- Large, gram positive (-ve or variable in old cultures)
- Majority are motile, but C. perfrengens is non-motile
- Anaerobic, rod shaped, spore forming (central, sub-terminal or terminal spores),
- Ferment organic compounds
  - Acid, alcohols, gas production during fermentation of sugars
  - Foul smelling products from fermentation of aminoacids and fats
- Produce extra-cellular enzymes
  - Degrade biological molecules
  - Role in invasion and pathogenesis
  - play an important role in nature in biodegradation and the carbon cycle
- Cause tetanus, botulism, pseudo-membranous colitis, food poisoning and gas gangrene
- Most are saprophytes
- Found in soils, aquatic sediments, skin, intestinal tract, and feces of animals
- Include four medically important Spp**:**
  - *C. botulinium, C. tetani, C. perfrigens* and C. difficile

Classification of Clostridia

- Based on shape and position of spore
- Based on biochemical properties
  - Predominantly saccharolytic Clostridia (eg.C. perfringens)
  - Predominantly proteolytic Clostridia (eg. C. butulinum A,B,F)
  - Slightly proteolytic Clostridia (eg. C. tetani)
  - Sacchrolytic Clostridia (eg. C. butulinum C,D,E)
  - Neither proteolytic nor saccharolytic Clostridia (eg, C. cochlearua)

Clostridium tetani

- Terminal spores within a swollen sporangium
  - Drumstick/ tennis racquet appearance
- Potent toxin-neurotoxin
  - Organism multiply locally and symptoms appear remote
- Most strains are motile (peritrichous flagella)
- Horse and humans are susceptible,
- cause tetanus

Virulence determinants

- Tetanus toxin/tetanospasmin
  - Plasmid encoded
  - Tropism for inhibitory motor neurons
  - Block the release of inhibitory neurotransmitters
  - Heat labile, O_2 labile

Pathogenesis

- Tetanus results from trauma or a puncture wound leading to tissue contamination.
- Tetanus is a non-invasive disease occurring because of the release of exotoxins.
- *C. tetani* produces a spasmogenic toxin that fixes to gangliosides thereby blocking the release of the neurotransmitter glycine.
- Glycine normally prevents contraction of antagonistic muscles; therefore, muscle spasms and convulsions (lockjaw) may occur.
- Cardiac failure can lead to death in approximately 55-65% of affected persons.

Clinical manifestation

- Neonatal and adult tetanus
- incubation period may range from 4–5 days to as many weeks
- The disease is characterized by tonic contraction of voluntary muscles.
- Muscular spasms often involve first the area of injury and infection and then the muscles of the jaw (trismus, lockjaw), which contract so that the mouth cannot be opened.
- Gradually, other voluntary muscles become involved, resulting in tonic spasms.
- Any external stimulus may precipitate a tetanic generalized muscle spasm.
- The patient is fully conscious, and pain may be intense.
- Death usually results from interference with the mechanics of respiration.
- The mortality rate in generalized tetanus is very high

Lab diagnosis

Specimen

- are usually wound swabs, or excised bits of tissue from necrotic depth of wound

Microscopy

- **Gram stain inwound: drum stick appearance**

Culture

- *C. tetani* is a strict anaerobe with a temperature range of 14–43 ºC (37 ºC optimum).
  - ***Blood agar:***
    - When isolated (only very occasionally), *C. tetani* produces a fine film of feathery growth.
    - Use a hand lens to examine the plate.
    - On fresh blood agar, *C. tetani* is haemolytic (*alpha* first followed by *beta* haemolysis).

Antitoxin test

- If growth occurs on blood agar, subculture on a blood agar antitoxin plate (half the plate covered with antitoxin).
- Incubate the plate anaerobically.
- The haemolysis produced by *C. tetani* is inhibited by the antitoxin.

Robertson's cooked meat medium (RCMM):

- *C. tetani* is slowly proteolytic.
- If clostridial growth occurs (check a Gram smear), divide the culture and heat one half at 80 ºC for 30 minutes and cool.
- Subculture both the unheated and heated cultures on fresh blood agar and incubate anaerobically.
- Culture in cooked meat broth produce blackening of meat and foul smelling product.

Prevention & control

- Active immunization with toxids
- Proper care of wounds contaminated with soil
- Prophylactic use of antitoxin
- Administration of penicillin

C. perfringens

- oval, sub-terminal and non-bulging spores
- Non-motile bacteria

Virulence factor:

- Exotoxin, Enterotoxin, and hydrolytic substance
  - Based on surface antigens and major lethal toxins (α,β,ε,Z) produced, five types of *C. perfringens* (A–E) are recognized.

Pathogenesis:

- Human disease is caused by types A and C (other types cause disease in animals).
- All types of *C. perfringens* produce *alpha* toxin.
- Type B and C strains also produce *beta* toxin.
- *C. perfringens type A1*: Causes gas gangrene (myonecrosis), anaerobic cellulitis, puerperal infection and septicaemia.
  - *Note*: Other species of *Clostridium* that occasionally cause gas gangrene include *C. novyi* and *C. septicum*.

- *C. perfringens type A2*: Causes food-poisoning, usually within 8–12 hours of ingesting contaminated meat.
- *C. perfringens type C*: Causes a severe form of jejunitis (necrotizing enterocolitis), known as pigbel.
  - The source of infection is usually insufficiently cooked pig meat.
  - The condition is often fatal especially in young children.

Clostridium perfringens

Clinical features

- Wound infection and gas gangrene/clostridial myonecrosis
  - the infection spreads in 1–3 days to produce
    - crepitation in the subcutaneous tissue and muscle,
    - foul-smelling discharge,
    - rapidly progressing necrosis,
    - fever, hemolysis, toxemia, shock, and death.

Lab diagnosis

- Specimen: consist of
  - material from wounds, pus, and tissue.
- Microscopic examination
  - The presence of large gram-positive rods in Gram-stained smears suggests gas gangrene clostridia;
  - spores are not regularly present.
- Culture
  - Material is inoculated into chopped meat-glucose medium and thioglycolate medium and onto blood agar plates
  - incubated anaerobically.
  - The growth from one of the media is transferred into milk.
  - A clot torn by gas in 24 hours is suggestive of *C perfringens.*

Biochemical tests

- Clostridia are catalase and oxidase negative.
- The most useful biochemical reactions which help to identify *C. perfringens* and other pathogenic *Clostridium* species can be tested by culturing the organism anaerobically on lactose egg yolk medium
- This medium tests for lecithinase C activity, lipase hydrolysis, lactose fermentation and proteinase activity.
  - **Lecithinase C activity:** Seen as an opacity in the medium due to the breakdown of lecithin in the egg yolk.
  - **Lipase hydrolysis:** Seen as a pearly (fatty) layer covering colonies and sometimes extending into the medium A restricted intense area of opacity is also produced in the medium.
  - **Lactose fermentation:** There is a reddening in the medium. The colonies become red on exposure to air.
  - **Proteinase activity (proteolysis):** Shown by an area of clearing around the colonies due to the breakdown of casein in the milk by the enzyme proteinase.

On lactose egg yolk medium, *C. perfringens*:

- Produces lecithinase C (*alpha* toxin)
- Ferments lactose
- Does not hydrolyze lipid
- Shows no proteinase activity

Nagler reaction

- Used for final identification of *C. perfringens*
- It is about toxin production and neutralization by specific antitoxin. The technique is referred to as the Nagler reaction
- In medium containing lecithin, the opacity that can be produced by *C. perfringens* can be inhibited by applying specific antitoxic serum to the medium which will inactivate the lecithinase.

C perfringens rarely produces spores when cultured on agar

Prevention & Control

- Early and adequate cleansing of contaminated wounds and surgical debridement, together with the administration of antimicrobial drugs directed against clostridia (eg, penicillin),

Treatment:

- Penicillin
  - Prompt and extensive wound debridement
  - Polyvalent antitoxin

C. butulinum

- Oval, sub-terminal endospores
- Motile (perithricous flgella)
- Spore, relatively heat resistant (survive improper canning and growth encouraged in this anaerobic environment)
- Grow best in neutral or low acid
- In decaying vegetation, intestinal tract of birds, mammals, sedments of lkes, ponds, soil.
- Types of *C botulinum* are distinguished by the antigenic type of toxin they produce.
- Spores of the organism are highly resistant to heat, withstanding 100 °C for several hours.
- Heat resistance characteristics is diminished at acid pH or high salt concentration.
- *C. botulinum* can be found in soil, water, and sewage.



Fig. Clostridium botulinum

Virulence factors

- Botulism toxin/ neurotoxin
  - it is heat labile
- Seven toxin types (A-G)/serotypes
  - Human illness is caused by mainly Type **A, B, & E, and rarely by F**
  - Type **E** linked with fish products
  - Type **A & B** associated with variety of foods
  - Type **D** cause botulism in mammals ----- important!!!
  - Types C and D are encoded by bacteriophage that infect the bacteria.

- Not all strains produce toxin
- Affect mainly peripheral nervous system
  - Prefer stimulatory motor neurons
  - Weak/ flaccid paralysis
- The toxin is formed in food when *C. botulinum* spores contaminate food.
- Under anaerobic conditions, e.g. in tinned meat or fish (not acid fruits), the spores germinate and the bacilli multiply,producing toxin.

Clostridium botulinum

Pathogenesis

Food-borne Botulism

- Botulism is not infection but an intoxication resulting from the ingestion of food in which *C. botulism* has grown (spores have germinated) & produced toxin
- rare and usually fatal
- Result from an ingestion of a lethal preformed neurotoxin, about 18-36 hours following ingestion of preformed toxin,
- These protein exotoxins are often released in an inactive form, then proteolytic cleavage activates them.
  - Type A is the most potent exotoxin known

- These toxins block the release of the neurotransmitter acetylcholine resulting in double vision, slurred speech, decreased saliva, difficult swallowing and general weakness.
- The toxin causes paralysis in about 20% of those affected.
- Death usually occurs from respiratory failure.

Infant butulism

- Rarely *C. botulinum* causes infantile botulism in which the bacteria colonize the gut of infants and produce toxin which is absorbed.

Wound botulism

- The bacteria growing in the necrotic tissue of the wound

Clinical findings

- Sign & symptoms begin: 18 - 36 hrs after ingestion of toxic food
- Visual disturbances (blurred vision)
- Difficulty to swallow
- Speech difficulty
- Gastrointestinal symptoms are not prominent
- No fever, dizziness and dryness of the mouth
- Descending weakness of skeletal muscles
- Death occurs from respiratory paralysis or cardiac arrest
- The patient remains fully conscious until shortly before death.

N.B. Patients who recover **do not** develop antitoxin in the blood

- In Infant botulism
    - constipation, weak sucking ability, generalized weakness occur in infants at 5-20wks of age

Lab diagnosis

- ***Specimens*:**
  - Include suspected food and patient's faeces and serum **(to demonstrate toxin).**
  - In infant botulism, *C botulinum* and toxin can be demonstrated in bowel contents but not in serum
  - *C botulinum* may be grown from food remains and tested for toxin production, but this is rarely done and is of questionable significance.
- Toxin may be demonstrated by passive hemagglutination or radioimmunoassay.
- The antigenic type of toxin is identified by neutralization with specific antitoxin in mice.

Culture and biochemical reactions

- *C. botulinum* is a strict anaerobe.
- Grows best at 30–35 ºC.
- ***Robertson's cooked meat medium (RCMM):***
  - Inoculate the emulsified specimen (in 0.1% peptone water) in several containers of RCMM.
  - Heat half of them at 80 ºC for 30 minutes (spores remain).
  - Incubate the heattreated and untreated inoculated RCMM at 35 ºC for 3–5 days.
  - Types A, B and F blacken and digest cooked meat medium (proteolytic reaction) and produce hydrogen sulphide gas (types C, D, and E do not).

- ***Blood agar subculture from RCMM (anaerobic culture):***
  - *C. botulinum* produces large semi-transparent colonies with a wavy outline.
  - Most strains are *beta*-haemolytic.
- ***Lactose egg yolk milk agar*:**
  - *C. botulinum* hydrolyzes lipid (pearly opalescence).
  - Types A, B, and F show proteinase activity (area of clearing around the colonies).
  - Lactose is not fermented

Prevention and control

- Spores of C. botulinum are widely distributed in soil,
- They often contaminate vegetables fruits and other foods
- When such foods are canned / preserved, they either:
    - must be sufficiently heated to destroy spores or
    - boiled for 20min before consumption
- Strict regulation of commercial canning
- Careful observation of the cans
  - **Eg.** Swelling of the cans

Treatment

- Trivalent (A, B, E,) anti - toxins are available
- Promptly administered intravenously

Clostridium difficile

- Slender bacilli - with large, oval, subterminal spores
- Members of the intestinal flora
- Nosocomial pathogen

Virulence factor

- Two toxin: Toxin A (enterotoxin), Toxin B (extremely lethal/cytopathic toxin)

Pathogenesis

- Pseudomembranous colitis
  - Although many antibiotics have been associated with pseudomembranous colitis, the most common are ampicillin and clindamycin.
  - Administration of antibiotics results in proliferation of drug-resistant *C difficile* that produces two toxins.
    - Toxin A, a potent enterotoxin that also has some cytotoxic activity, binds to the brush border membranes of the gut at receptor sites.
    - Toxin B is a potent cytotoxin.
    - Both toxins are found in the stools of patients with pseudomembranous colitis.
    - Not all strains of *C difficile* produce the toxins, and the *tox* genes apparently are not carried on plasmids or phage.

- Antibiotic-Associated Diarrhea
  - The administration of antibiotics frequently leads to a mild to moderate form of diarrhea, termed antibiotic-associated diarrhea.
  - This disease is generally less severe than the classic form of pseudomembranous colitis.
  - As many as 25% of cases of antibiotic-associated diarrhea may be associated with *C difficile.*

Clinical feature

- mild to moderate form of diarrhea, termed antibiotic-associated diarrhea
- Plaques and microabscesses may be localized to one area of the bowel.
- The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever

Lab diagnosis

- detection of one or both *C difficile* toxins in stool
- endoscopic observation of pseudomembranes or microabscesses in patients who have diarrhea and have been given antibiotics.

Treatment

- The disease is treated by discontinuing administration of the offending antibiotic and orally giving either metronidazole or vancomycin.

Genus Corynebacterium

- non-spore-forming gram-positive bacilli.
- *Corynebacterium* species tend to be clubbed or irregularly shaped
- These bacteria have a high guanosine plus cytosine content
- Are members of the normal flora of skin and mucous membranes of humans.
- Other corynebacteria are found in animals and plants.

- Comprises the toxin-producing pathogen C. diphtheriae, C. ulcerans and several commensals (diphtheroides) which normally colonize the skin, nasopharynx, oropharynx, GIT and UGT.
- *Corynebacterium diphtheriae* is the most important member of the group, as it can produce a powerful exotoxin that causes diphtheria in humans.

Corynebacterium diphtheriae

- *C. diphtheriae* is non-capsulate, non-motile, and does not form spores.
- Characteristically, they possess irregular swellings at one end that give them the "club-shaped“f appearance
- Irregularly distributed within the rod (often near the poles) are granules staining deeply with aniline dyes (metachromatic granules) that give the rod a beaded appearance.
- Individual corynebacteria in stained smears tend to lie parallel or at acute angles to one another.
- True branching is rarely observed in cultures.
- Arranged in angular fashion like Chinese lettering or cuneiform arrangement

C. diphtheriae biovars

- There are four biovars (biotypes):
  - *gravis*, *intermedius*, *mitis*, and *belfanti*.
- Originally these names were used to describe the severity of disease.
- It is now known that toxigenic and non-toxigenic strains occur in all *C. diphtheriae* biovars.
- In the investigation of diphtheria, it is not necessary to differentiate these biovars.

Virulence factor:

- All toxigenic *C diphtheriae* are capable of elaborating the same disease-producing exotoxin.
- Diphtheria is caused by toxin-producing strains of *C. diphtheriae*.

Pathogenesis

C. diphtheriae causes:

- Nasal, nasopharyngeal and tonsillar diphtheria, especially in young children.
  - Often there is marked oedema of the neck.
  - Infection is by inhaling respiratory droplets.
- Cutaneous (skin) diphtheria
  - Usually develops when *C. diphtheriae* infects open wounds.
  - Infection of the skin rarely leads to the serious complications associated with diphtheria of the throat.

Lab. Diagnosis:

***Specimens*:**

- Include throat, and, or nasopharyngeal swabs to confirm a diagnosis of throat diphtheria, and a skin swab if cutaneous diphtheria is suspected

Microscopy:

- *C. diphtheriae* is Gram positive but usually stains unevenly and weakly.
- It is markedly pleomorphic.
- Long, thin, and curved forms can be seen
- short rods and rods enlarged at one end (clubshaped).
- They often appear in clusters, joined at angles like Chinese letters

- In **Albert stained smears**, particularly from Loeffler serum or Dorset egg cultures, *C. diphtheriae* often appears beaded due to the presence of darkstaining granules in the rods
  - These granules, known as volutin or metachromatic granules, are energy-storing inorganic polyphosphate units.
  - In some strains the granules form at the ends of the rods.
- In **toluidine blue stained smears**, the organisms stain pale blue and the granules dark red-purple.

Culture

- *C. diphtheriae* is an aerobe and facultative anaerobe.
- Temperature range for growth is 20–40 ºC with an optimum of 35–37 ºC.

***Loeffler serum medium and Dorset egg medium*:**

- *C. diphtheriae* grows rapidly on these media, producing significant growth in 4–6 hours.
- The characteristic morphological features of *C. diphtheriae*, especially granule formation, are well developed.

***Tellurite blood agar*:**

- This medium is widely used as a primary medium for isolating *C. diphtheriae* from throat and nasopharyngeal swabs.
- *C. diphtheriae* reduces tellurite and produces grey or grey-black colonies after 24–48 h incubation.
- Commensal diphtheroid colonies are grey, non-haemolytic,

Tinsdale medium:

- After 24–28 h incubation,*C. diphtheriae* colonies are grey-black, raised, and surrounded by a dark brown area.
- The brown colour is due to the hydrogen sulphide produced from the cystine interacting with the tellurite.
- Occasionally commensal diphtheroids and other respiratory tract commensals may grow on Tinsdale’s medium but the colonies are not surrounded by a brown halo like those of *C. diphtheriae*.

Biochemical tests

- All *C. Diphtheriae reduces* nitrate to nitrites
- *Toxigenic strain of C. Diphtheriae is*:
  - Catalase and.
  - Oxidase negative.
  - Urease negative.
  - Ferments glucose and maltose with acid production
- A few strains of *gravis* and *mitis* biovars ferment sucrose.
- *C. diphtheriae gravis* ferments starch with acid production.

Toxigenicity (virulence)

- Toxigenicity of *C. diphtheriae* can be tested by the Elek gel precipitation test.
- ***Elek gel precipitation test method***
  - A rectangular strip of filter paper soaked in antitoxin is placed on the surface of a serum agar plate containing 20% horse serum while the medium is still in fluid form.
  - When the medium solidifies, the testing strain is streaked across the plate at the right angles to the filter paper strip and then incubated as 37^0C for 24-48hrs.
  - Toxin produced by bacterial growth diffused in agar medium and produces line of precipitation where it meets antitoxin molecules (dispersed from the filter paper) in optimum concentration.
  - The line of precipitation radiates from the intersection of the strip and the bacterial growth.
  - *Toxin-producing C. diphtheriae* is identified by the presence of precipitin lines and arcs of identity

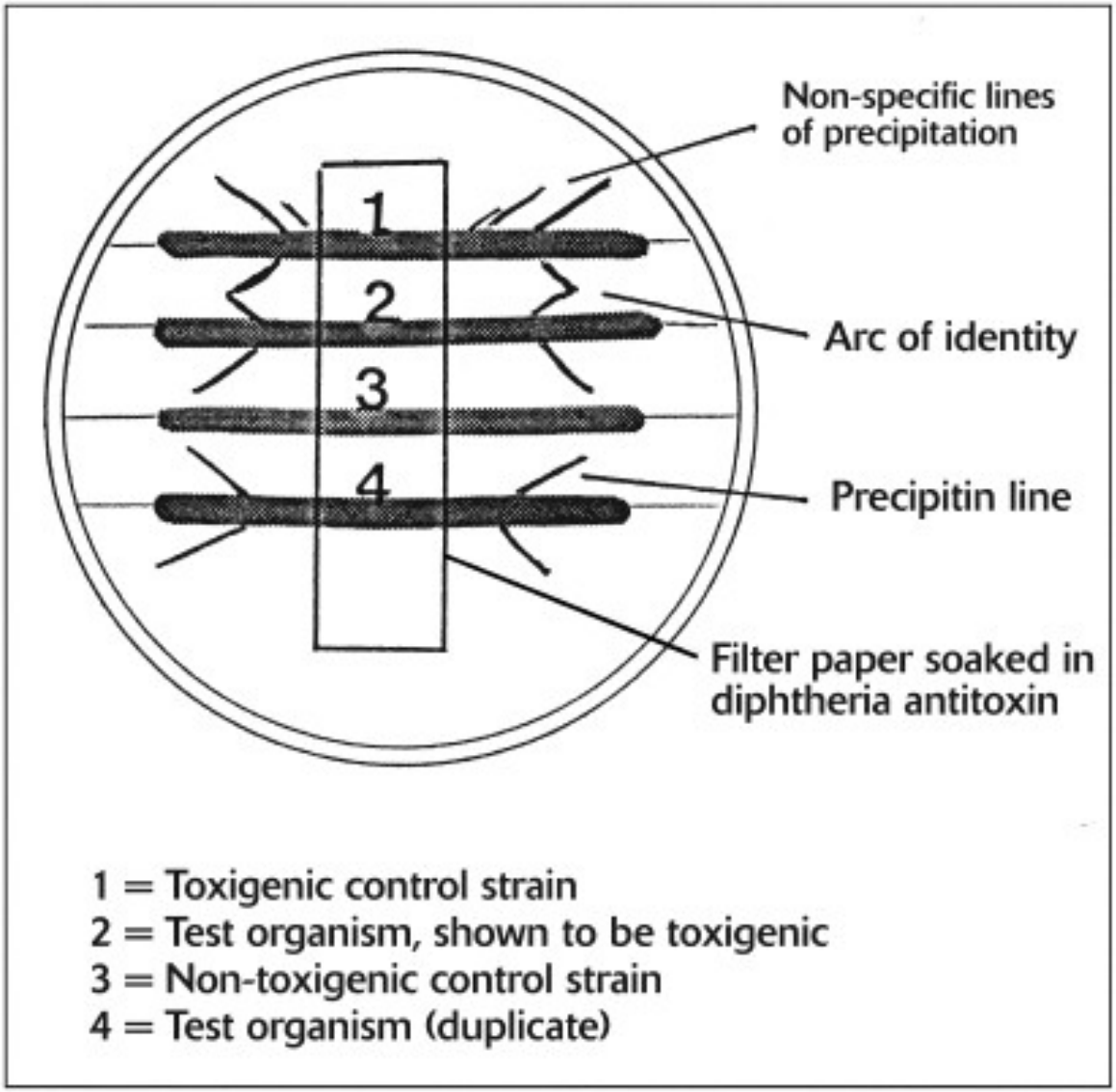


Fig. Elek plate toxigenicity test.

Treatment

- The treatment of diphtheria rests largely on rapid suppression of toxin-producing bacteria by
  - antimicrobial drugs (Penicillin or Erythromycin)
  - early administration of specific antitoxin against the toxin formed by the organisms at their site of entry and multiplication.
    - Diphtheria antitoxin

Prevention and control

- Active immunization
- Passive immunization
- Combined immunization