MICROBIOLOGY LAB . 7

Gram- positive rods
Non-spore formers

*Corynebacterium* & *Listeria*

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Learning Objectives:

After this lab, you must be able to:

- Distinguish between *Corynebacterium* and *Listeria*
- Describe the two genera microscopically and culturally.
- List types of clinical infections these organisms produce.
- Predict G+ve causative agents causing clinical cases.
- Discuss the principles of identifying tests.
- Know the prevention ways of each organism.
Non spore-forming Gram-positive bacilli

There are two important pathogens in this group:

- *Corynebacterium diphtheriae*
- *Listeria monocytogenes*
Corynebacterium diphtheriae
Important properties:

- G +ve rods, that appear **club-shaped**, arranged in **palisade** or in **V** or **L** shaped Chinese letters.
- Have **metachromatic granules**.
- Non motile, non sporing, and non capsulated.
- **Pleomorphic**.
Gram stain of diphtheroids. The term ‘diphtheroids’ includes various Corynebacterium species that are skin commensals. The Gram stain shows a typical ‘Chinese lettering’ arrangement of the bacilli. (Gram stain, ×1000)
Clinical findings:

- **Respiratory diphtheria**: Thick, gray, adherent pseudomembrane over the tonsils and throat.
- **Cutaneous diphtheria**: Ulcerating skin lesion covered by a gray membrane.
Laboratory diagnosis:

- **Specimens:** throat swab or swab from pseudomembrane
- **Microscopy:**
  - Staining by Gram or methylene blue (G +ve rods arranged as L or V shaped)
  - Albert stain: differential stain for metachromatic granules.
- **Culture:**
  - Loeffler’s serum slope: creamy white colonies in 6–8 hrs.
Laboratory diagnosis:
- Potassium tellurite medium: black colonies.

This agar contain tellurium salt that is reduced to elemental tellurium within the organism.

- **Toxigenicity test:**
  1- *in vivo*: animal inoculation, Schick test.
  2- *in vitro*: (Elek’s test) antibody-based gel diffusion precipitation test to confirm toxin production.
  3- tissue culture neutralization assay.
  4- **PCR assay**: for the presence of toxin gene in the organism isolated from the patient.
Schick test:

Intracutaneous skin test distinguishes between persons who are susceptible and those who are immune to diphtheria toxin and to test for sensitivity to toxoid.

Procedure: the test is performed by intradermal injection of 0.1 mL of purified standardized toxin, if the patient has no antitoxin the toxin will cause inflammation at the site of injection 4-7 days later. If no inflammation occurs, antitoxin is present and the person is immune.
Tellurite blood medium

143 *Corynebacterium diphtheriae*, tellurite blood medium. *Corynebacterium diphtheriae* reduces tellurite and produces gray-black colonies. Commensal diphtheroids are gray. (Tellurite blood agar, 48 h at 37°C)
Elek Test

sterile filter paper with \textit{C. diptheriae} antitoxin

Ab-Ag precipitation line

bacteria
Elek’s test:

146 Elek plate to demonstrate the toxigenicity of Corynebacterium diphtheriae. The filter paper strip contains diphtheria antitoxin; it is placed in the Petri dish and the medium is poured on. The test strain and toxigenic and non-toxigenic strains are inoculated at right angles to the strip. A toxigenic strain produces a V-shaped line of precipitation between the toxin and anti-toxin. (Elek’s medium, 48 h at 37°C)
Treatment and prevention:

- The treatment of choice is antitoxin. The decision to treat with antitoxin is a clinical one and cannot wait for the laboratory results.
- Penicillin G or erythromycin.

- The disease is prevented by diphtheria vaccine (DTaP).
**Listeria monocytogenes:**
Causes meningitis and sepsis in **newborns, pregnant women, and immunocompromised patients.**

**Important properties:**

1- small, G +ve rods arranged in L or V shape similar to *Corynebacterium*

2- β-hemolytic

3- motile with tumbling movement at room temp.

4- grow well and multiply at refrigerator temp. (cold enhancement).

5- CAMP test +ve
Laboratory diagnosis:

- **Specimens:** CSF and blood
- **Microscopic:** Gram stain, CSF typically shows no *Listeria* because of the low bacterial concentration.
- **Culture:**
  Mueller-Hinton agar + sheep blood: produce a narrow zone of β-hemolysis
  
  *Isolation can be enhanced if the specimen is kept at 4ºC for some days before inoculation into media*

  **Note:** the motility at room temp. and the hemolysin production are primary findings that help in differentiation of *Listeria* from *Corynebacterium.*

- **Identification** is made by sugar fermentation tests and serology