Gram positive Rods Lab:5

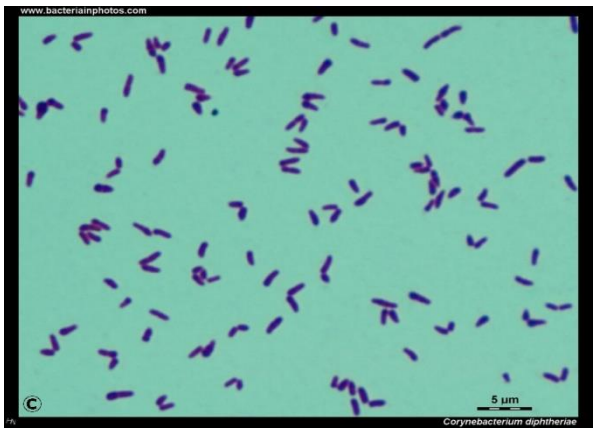
CORYNEBACTERIA

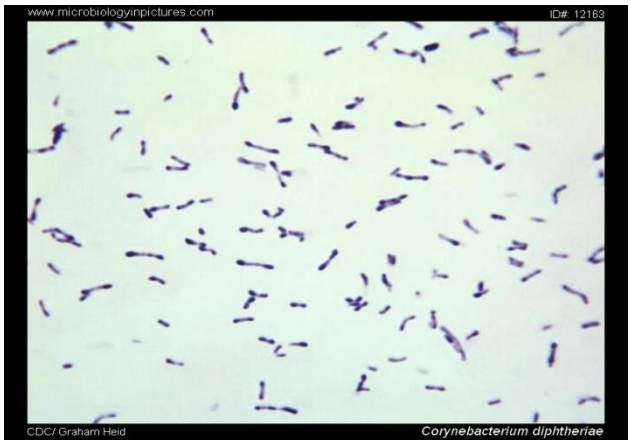
LABORATORY FEATURES

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

Morphology

C. diphtheriae is Gram positive but usually stains unevenly and weakly. It is markedly pleomorphic. Long , thin, and curved forms can be seen and also short rods and rods enlarged at one end (club shaped). They often appear in clusters, joined at angles like Chinese letters Commensal diphtheroids: These are strongly Gram positive and stain uniformly. They are usually short and show little variation in size and form .



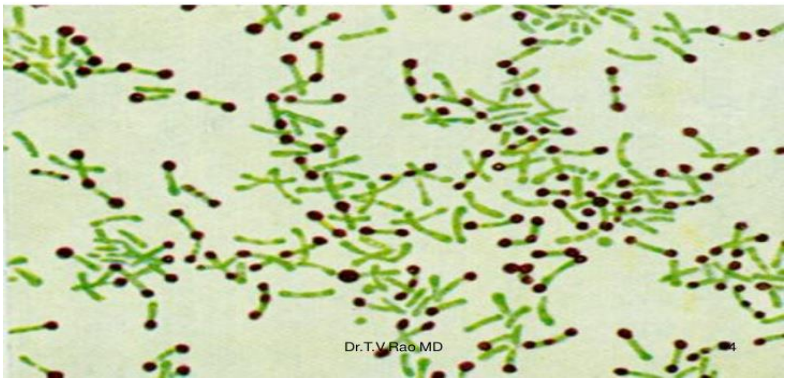


Volutin granules

In Albert stained smears, particularly from Loeffler serum or Dorset egg cultures, *C. diphtheriae* often appears beaded due to the presence of dark staining granules in the rods.

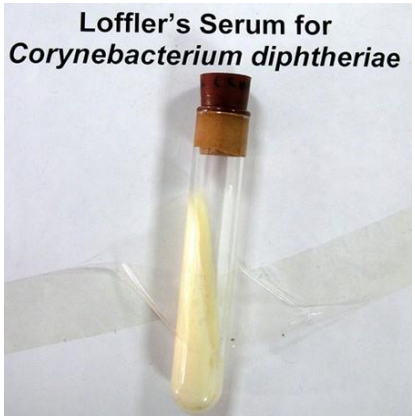
These granules, known as volutin or metachromatic granules, are energy-storing inorganic polyphosphate units. In some strains the granules form at the ends of the rods. In toluidine blue stained smears, the organisms stain pale blue and the granules dark red-purple.

Corynebacterium diphtheria



Culture

***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 .



Note: It is not advisable to use either Dorset egg or Loeffler serum medium as a primary medium for isolating *C. diphtheriae* because commensal diphtheroids may overgrow the diphtheria bacteria.

***Tellurite blood agar*:** This medium is widely used as a primary medium for isolating *C. diphtheria* from throat and nasopharyngeal swabs. *C. diphtheriae* reduces tellurite and produces grey or grey-black colonies measuring 0.5–2 mm in diameter after 24–48 h incubation .

Tinsdale medium: After 24–28 h incubation, *C. diphtheriae* colonies are grey-black, raised, and surrounded by a dark brown area .

Biochemical tests

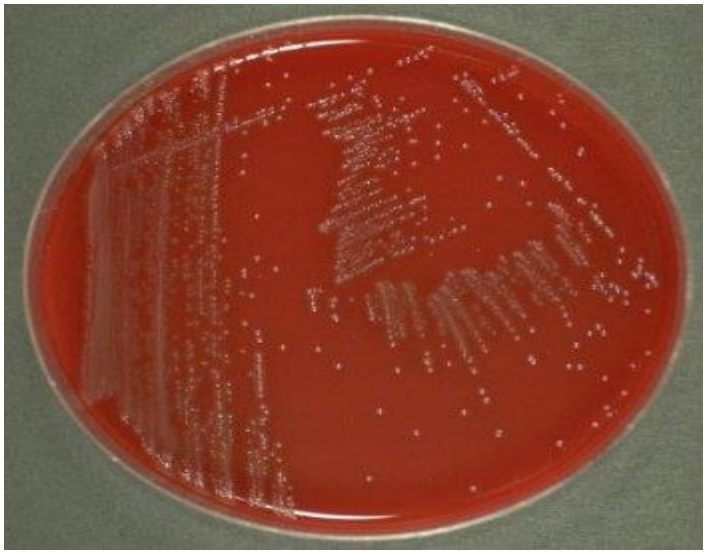
- Catalase and nitrate positive.
- Oxidase negative.
- Urease negative

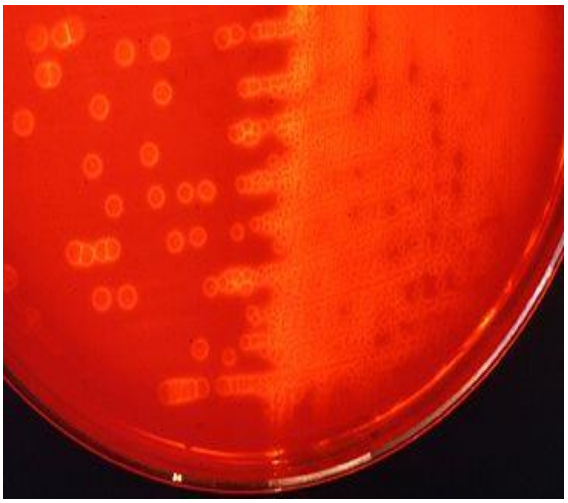
Listeria

Laboratory identification

The organism can be isolated from blood, cerebrospinal fluid, and other clinical specimens by standard bacteriologic procedures. On blood agar, *L. monocytogenes* produces a small colony surrounded by a narrow zone of β hemolysis

Listeria species can be distinguished from various streptococci by morphology, motility, and the production of catalase .



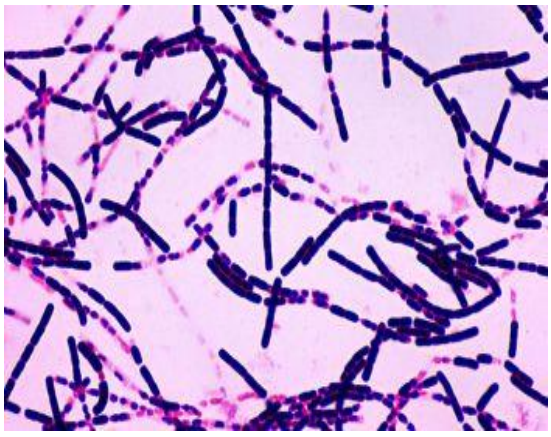


BACILLUS SPECIES

DIAGNOSIS

Caution: *B. anthracis* is a high risk infectious pathogen, therefore handle specimens and infected material with care, wearing protective gloves and face mask, and following recommended safety procedures. **Use 4% v/v formalin** solution to decontaminate infected material and laboratory ware.

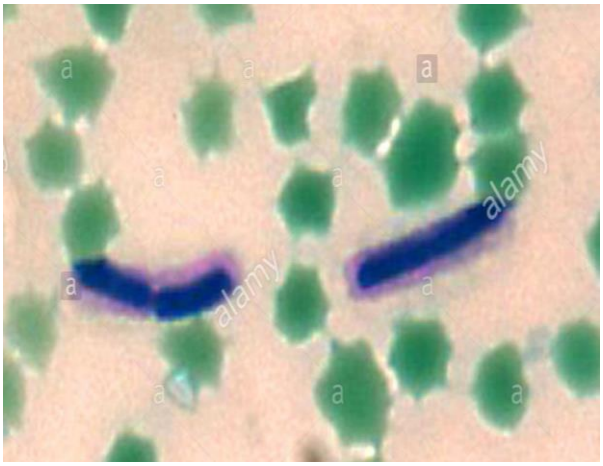
Sample: skin lesions, sputum, blood, and CSF are the primary means of anthrax diagnosis.



Morphology

Gram positive (or Gram variable) non-motile bacillus, often appearing joined end to end in chains.

In smears from specimens: Bacilli are capsulated. The capsular material often appears irregular and fragmented. When stained using Loeffler's polychrome (McFadyean) methylene blue, the bacilli stain blue and the capsular material stains purple-pink. Alternatively, **Giesma stain** can also be used when MacFadyean methylene blue is not available.



In smears from aerobic cultures: Bacilli are non-capsulated but contain oval spores (same diameter as the bacilli), giving the organisms a beaded appearance.

Fixation of smears

B. anthracis is not killed by heat-fixation. Smears should be chemically fixed by immersing the dry smears in a container of **potassium permanganate** 40 g/l solution for 10–15 minutes.

Important: When organisms resembling *B. anthracis* are seen in smears, specimens should be sent for further testing to the nearest Public Health Laboratory and public health officials notified as soon as possible

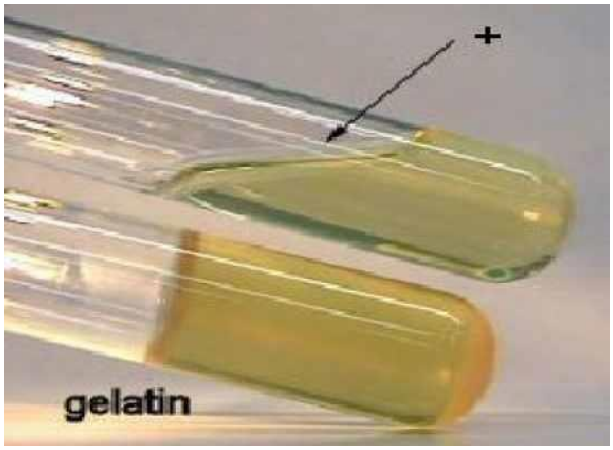
Culture

B. anthracis grows aerobically and anaerobically (facultative anaerobe). The temperature range for growth is 12–45 ºC with an optimum of 35–37 ºC. Spore formation is best between the range 25–30 ºC.

Blood agar: *B. anthracis* produces large 2–5 mm in diameter, grey-white, irregular colonies with wavy edges. The colonies are nonhaemolytic or only slightly haemolytic. The saprophytic species are usually β-hemolytic and motile; these features can be used to exclude *B. anthracis.*

Gelatin stab culture

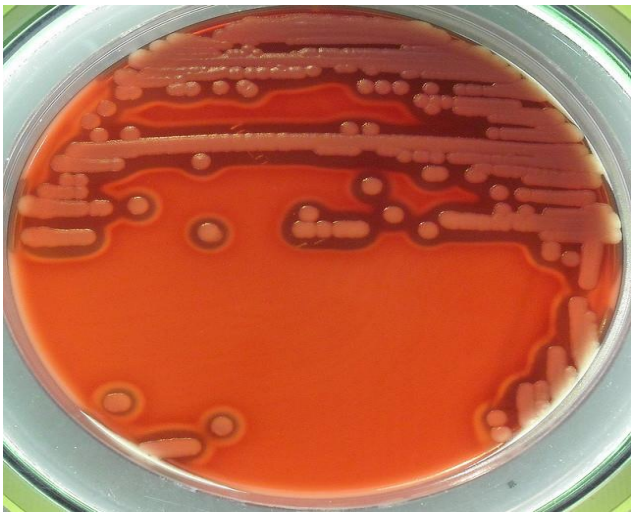
The organism slowly liquefies the gelatin.



B. cereus

B. cereus unlike *B. anthracis* is motile, non-capsulate, and produces haemolytic colonies on blood 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.



Bacillus cereus