Practical No.12

Corynebacterium

Corynebacterium is a genus of Gram-positive rod-shaped bacteria. They are widely distributed in nature. They are catalase positive, non-spore-forming, non-motile, rod-shaped bacteria that are straight or slightly curved. Metachromatic granules are usually present representing stored phosphate regions. Their size ranges from 2 – 6 µ in length and 0.5 µ in diameter. The bacteria group together in a characteristic way, which has been described as the form of V or Y shape, or what is called "Chinese letters arrangement". The most notable human infection is diphtheria, caused by ***Corynebacterium diphtheriae*.** It is also known as the Klebs-Löffler bacillus. The bacteria which are only lysogenic for phage β are capable of producing a powerful cytotoxin. Production of this toxin leads to the formation of pseudomembranes which are composed of dead epithelial cells, dead and live white blood cells, red blood cells, and fibrin that form around the tonsils and back of the throat, which may lead to suffocation of the infected child.

Laboratory diagnostic tests;

Specimen; Smear from a throat swab from a diphtheria case;

1- **Gram stain;** is performed to show gram-positive, highly pleomorphic organisms with no particular arrangement (classically resembling Chinese letters).

Special stain like **Alberts's stain** is used to demonstrate the metachromatic granules (or polyphosphate, or Babes-Ernst granules). Fixed smear is prepared, Albert's stain is added for 3-5 minutes, washed with tap water, lugol's iodine is applied for 1 minute, washed then dried and finally examined under the microscope. . The cytoplasm appears light green and the granules blue/black.

2- **Culture;** culturing the organism on an enrichment medium;

A- Löffler's serum, is used primarily for the cultivation of corynebacteria.This is a firm coagulated serum medium containing nutrient broth, prepared as slants. It is a selective medium, does not enrich other oraganisms of the throat. K.L.B. appears small, circular, grey, opaque disks with regular borders.

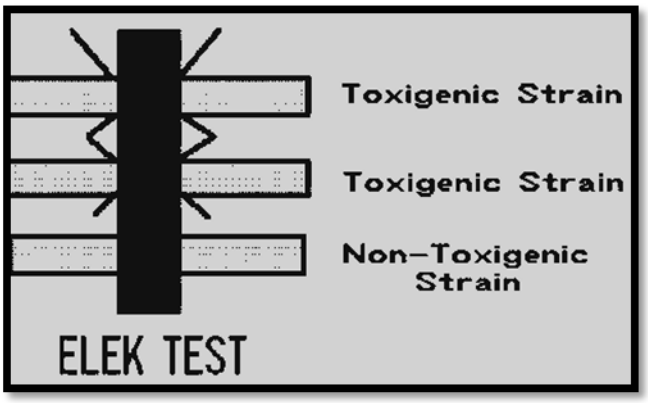
II- Tellurite agar, (blood agar + potassium tellurite); It is a selective medium which allows all *Corynebacteria* (including ***C. diphtheriae*)** to reduce tellurite to metallic tellurium producing brown colonies and, only in the case of *C. diphtheriae*, a black halo appears around the colonies allowing for easy differentiation of the organism.

III- Tinsdale medium; It contains cystine–tellurite, this medium is light yellow , colonies of ***C. diphtheriae*** appear Small, brownish-black colonies surrounded by a brown halo in the agar.

IV- Blood agar assures the recovery of corynebacteria as well as any other pathogenic bacterial species that might be present, differentiating those that may be hemolytic.

In vivo and in vitro tests

- **Elek's test for toxigenicity;** It is an in vitro test performed only in reference to public health laboratories in order to know if the organism is able to produce the diphtheria toxin or not. Filter paper strep containing antitoxin is placed on agar plate. The tested culture is streaked across the plate .after 48 hours the antitoxin precipitates the toxin, resulting in the formation of bands between the filter paper and the bacterial growth.



- **In vivo Schick's test**; intradermal injection of 0.1 ml of purified toxin. If a person does not have enough antibodies to neutralize that toxin, the skin around the injected area will become red and swollen, indicating a positive result. This swelling disappears after a few days. If the person has immunity, then little or no swelling and redness will occur, indicating a negative result.