Practical Microbiology - Lecture 7
Biochemical Tests

1) Catalae Test:

This test is can be used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H2O2) accumulation, which can occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. Catalase breaks H2O2 down into water and O2.

Method:

On the surface of clean slide mix few colonies with a drop of hydrogen peroxide, the formation of bubbles indicate a positive test.

It is an essential test to differentiate between genus of *Staphylococcus* and *Streptococcus, Staphylococcus* gives a positive result while *Streptococcus* gives a negative result.

2) Oxidase Test:

Oxidase enzyme play an important role in the operation of the electron transport system during aerobic respiration.

Method:

Few drops of freshly prepared oxidase reagent (1% tetramethyle –para-phenylene diamine dihydrochloride) are added to a piece of filter paper. A colony from tested organisms is transferred to the filter paper and rubbed onto the reagent using wooden stick. A positive reaction is indicated by blue purple color formation within 1 minuts, and a negative reaction by absence of coloration or coloration later than 60 seconds.

This test is a useful in differentiation of *Enterobacteriaceae* from *Pseudomonas* (*Neisseria, vibrio, and pseudomonase)* are oxidase positive.

3- Clumping Factor and Coagulase Test:

Used to differentiate *S. aureus* from other species of genus *Staphylococcus.*

Clumping Factor: is a surface compound that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma form the clumps. Detection of clumping factor is performed by emulsify the bacterial colony in a drop of saline on a clean slide to produce a dense uniform suspension, then mixed with a drop of undiluted plasma, a positive reaction is indicated by clumping or aggregation of bacteria with 5-10 seconds.

Coagulase test: coagulase is an enzyme act on fibrinogen (soluble plasma protein) and converted it into fibrin (insoluble). This test is performed by tube method by emulsifying a colony of tested bacteria in 1ml of diluted plasma 1:6 dilution of saline, incubated in 37°C, then examined after (1-4) hours, a positive result indicated by the clot formation as a result of

conversion the fibrinogen to fibrin, while the negative tubes re-examined after 24 hours.

4) Manitol Fermentation:

Mannitol is a polysaccharide that is fermented by some bacteria; mannitol semi solid media is prepared in test tubes with adding bromothymole blue as an **indicator**

Principle:

To test the ability of an organism to produce acid end products from mannitol fermentation. Used in identification of *Staphylococcus aureus* which give **positive** test.

Method:

The bacterial samples is inoculated into the media and the media incubated at 370C for 18 hours, if the sample contain bacteria that fermented mannitol the acid is formed as a results of fermentation, this acid decrease the pH of the media and this will change the color of the indicator from blue to yellow.