Lab 10- Gram Positive Cocci- Staphylococcus

Staphylococcus

The genus : *Staphylococcus* are part of the body normal flora . They are particulary numerous on the skin and in the upper Respiratory tract , including the anterior nares as well as pharyngeal surfaces some are also associated with human infection diseases. Staphylococci are gram positive cocci , characteristically arranged in irreular clusters.

Two Principal species

● ***Staphylococcus epidermidis*** : is usually not associated with pathogenicity .

● ***Staphylococcus aureus*** : The pathogenic strain of this genus and is resposible for bolis , abscesses , otitis media , carbuncles and similar sappurative processes in man.

Pathogenicity of *staphyloccocus . aureus* can be determined by

1-The ability of lysing R.B.Cs (Hemolysis)

2- The ability of lysing coagulating plasma by production of enzyme coagulase.

3- The ability of liquefying gelatine by the production of enyme gelatinase .

4- The ability of fermenting mannitol .

Microscopic characteristic:-

* All staphylococci appear gram- positive cocci occure singly in pairs , in short chain and most commonly in grape- like clusters .

* They are non- motile , non- spore forming aerobic or faculative anaerobe.

* Colony 1-2 mm in diameter , raised , conave , opaque with smooth surface and enter margine

* Colonies appear colored or pigmented as follows :

Staph aureus : golden – yellow colonies

Staph. Epidermides : white colonies

Staph . saprophyticus : white colonies .

Lab diagnosis :

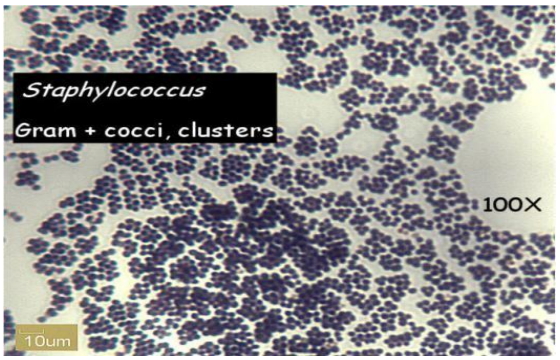
1- Specimen:

a- Pus(from abscess , osteomyelitis , Otitis media , Wound infection)

b- Urine (from cases of Urinary tract infection)

c- Blood (from cases of septicemia)

d- sputum (case of Lower Resiratory tract R.T)



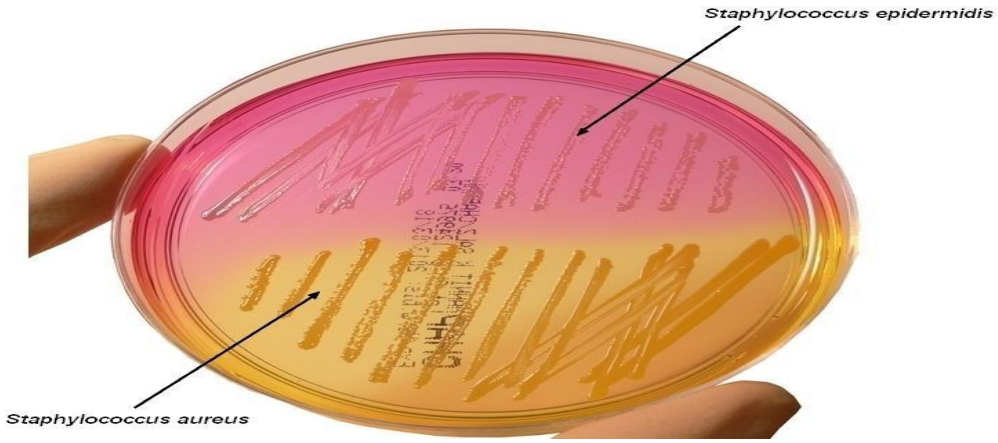
2- Microscopic :-

Gram- Stain smear (gram positive cocci , grape-like clusters (Staphylococci)

3- Culture :On blood agar and mannitol – salt agar

◘ **Blood agar Haemolysis (** beta – hemolysin) in *Staphylococcus . aureus* in *Stahylococcus epidermidis* (No hemolysis) . beta – hemolysis appear as clear zone around the colonies .

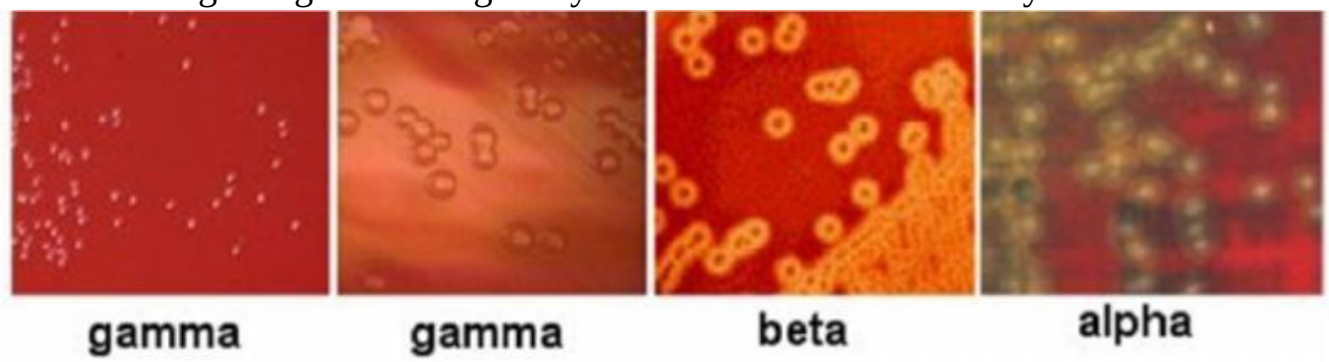
◘ **Manitol salt agar** (selective and fifferential medium) include Mannitol + 7.5% Nacl + henol red (indicator) + agar . The only Stahylococci can grow on this medium while other bacteria inhibited (this is due to the resistance to high salt concentration). The color of the medium is red in alkaline (befor fermentation and yellow in acidic (after fermentation) . ***Staphylococcus aureus*** produces yellow colonies with yellow zones, whereas other coagulase-negative staphylococci produce small pink or red colonies with no colour change to the medium.



Hemolytic Reaction

Blood agar (BAP) is a common medium used to culture bacteria because 1) it is a great enrichment medium for **fastidious** bacteria, and 2) hemolysis of blood cells can be very useful as an identification test. Blood agar is made with 5% sheep blood.

Hemolysis is the breakdown of red blood cells: hemolysins are enzymes produced by some bacteria and are released into the medium around the bacterial colony. It can be a complete breakdown of the cells, with the release of hemoglobin and a clearing of the red from the surrounding medium around the colony. Or the hemolysis can be a partial breakdown, resulting in a greenish or green-yellow zone around the colony.



- **alph (α)hemolysis -** green zone around colony, caused by leaking – hemoglobin converted to biliverdin, called a partial hemolysis

- **beta(β) hemolysis-** complete clearing around colony caused by breakdown of RBCs by streptolysin enzymes.

. - **gamma(ɤ)** hemolysis- no hemolysins, no zone

Biochemical reactions:

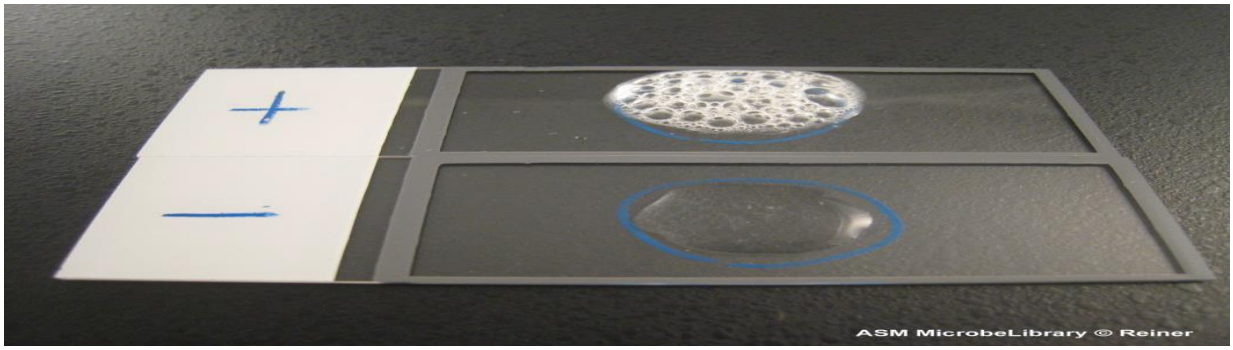
1- Catalase Test

Some bacteria contain flavoproteins that reduce oxygen (O2), resulting in the production of hydrogen peroxide (H2O2). Accumulation of these substances will result in death of the organism as they are powerful oxidizing agents and destroy cellular constituents very rapidly unless they can be enzymatically degraded. Many bacteria possess enzymes that afford protection against toxic H2O2 products, either *catalase* or *peroxidase*, which catalyze the destruction of hydrogen peroxide as follow

$$2H_2O_2 \xrightarrow[\text{Peroxidase}]{\text{Catalase}} O_2 + 2H_2O$$

Procedure of catalase test (Slide Test)

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
2. Place a drop of 3% H2O2 on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling. 4. A negative result is no bubbles or only a few scattered bubbles.



2- Coagulase Test

Short review:

This test is used to differentiate ***Staphylococcus aureus*** (positive) from Coagulase negative staphylococci (negative). ***S. aureus*** produced two forms of Coagulase:

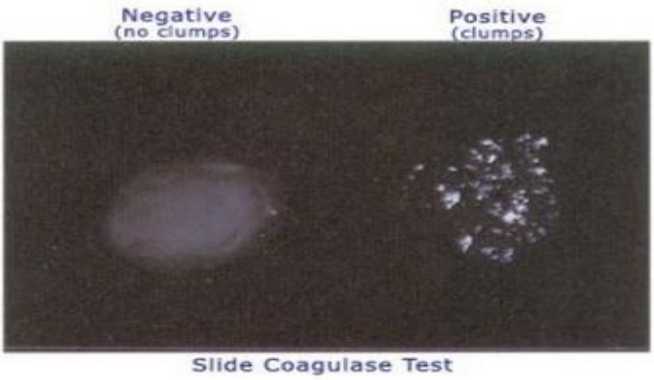
There are two methods for Coagulase test to be carried out:

1- Slide method.(slide Coagulase test for free clumping factor only)
2- Tube method.(tube Coagulase test for free and bound clumping factor)

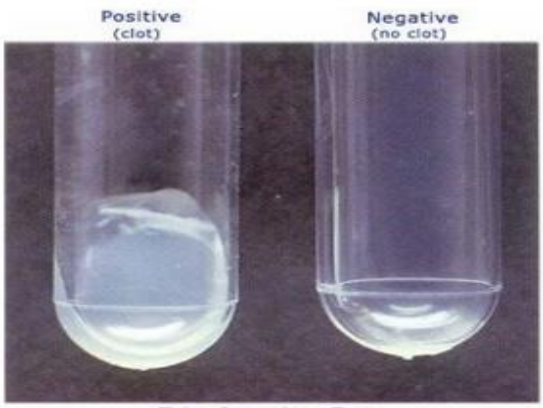
The Slide coagulase test

Procedure:

1. Divide the slide into two sections with grease pencil. One should be labeled as "test" and the other as "control.
2. a small drop of distilled water on each area.
3. Emulsify one or two colonies of Staphylococcus on blood agar plate on each drop to make a smooth suspension.
4. The test suspension is treated with a drop of citrated plasma and mixed well with a needle.
5. Do not put anything in the other drop that serves as control. The control suspension serves to rule out false positivity due to auto agglutination.
6. Clumping of cocci within 5-10 seconds is taken as positive.
7. Some strains of S.aureus may not produce bound coagulase, and such strains must be identified by tube coagulase test.



Slide Coagulase Test



Tube Coagulase Test