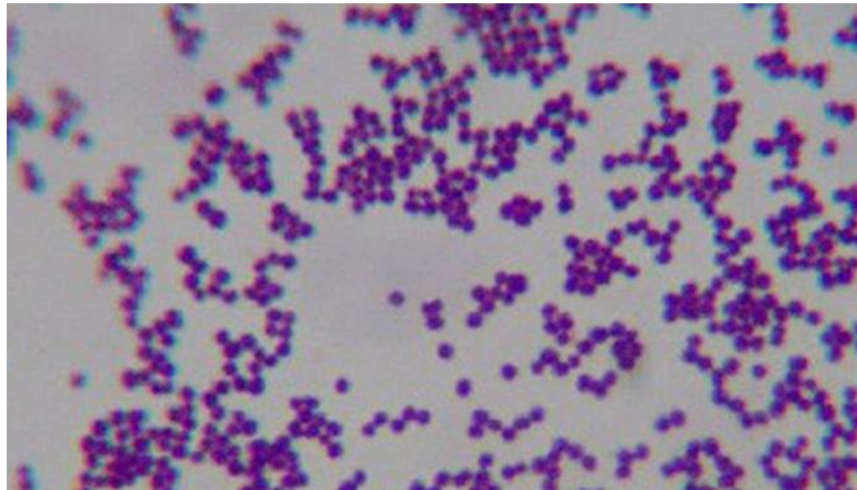


## ***Staphylococcus***

*Staphylococcus* is gram positive , non motile non spore forming, occasionally capsulate. Aerobic or facultative anaerobic.



### **Morphology and culture Characters:-**

*Staphylococcal* cells are spherical about  $1\mu\text{m}$  in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Micrococcus species often resemble *Staphylococci*, they are found free-living in the environment and form regular packets of four or eight cocci. Colonies on solid media are round, smooth, raised, and glistening. *S. aureus* (coagulase positive) usually forms gray to deep golden yellow colonies. Coagulase negative *Staphylococcus* like *S. epidermidis* colonies usually are gray to white, though some may be slightly pigmented, usually cream or yellow like *S. saprophyticus* colonies on primary isolation.

*S. aureus* produced Alpha-toxin that causes wide zone of clear (beta – type) hemolysis on blood agar. In rabbits it causes local necrosis and death.

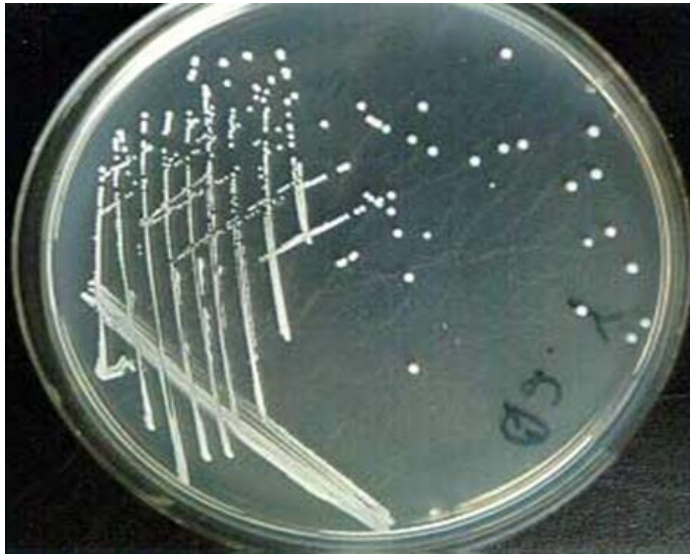
The other two species lack alpha toxin (do not exhibit hemolysis and are coagulase – negative).

## **Pathogenecity of Staphylococcus aureus:** •

- 1- The ability of lysis RBCs (hemolysis) by •  
producing hemolysin enzyme.
- 2- The ability to coagulate plasma by the •  
production of the enzyme coagulase which  
catalyses the transformation of fibrinogen to  
fibrin.
- 3- Other enzymes produced by staphylococci •  
include staphylokinase (cause fibrinolysis);  
proteinases; lipases and  $\beta$ -lactamase.
- 4- Ability to produce different types of toxins. •

## **Biochemical tests:- •**

- 1- catalase positive •
- 2-oxidase negative •
- 3- coagulase positive (slide and tube method) •
- 4- gelatinase positive •
- 5- fermentation of mannitol. •
- 6- beta-hemolysis.
- 7-motility negative
- 8-indole negative
- 9-MR (Methyl Red) positive
- 10- vp (Voges proskauer ) positive
- 11-Citrate positive



### **Clinical findings**

A localized staphylococcal infection like abscess, hair follicle infection, wound infection, Food poisoning and chronic osteomyelitis, meningitis following skull fracture, if *staphylococcus* disseminates can cause bacteremia and endocarditis.

### **Specimens**

Pus, blood, urine, surface swab, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

## **Lab. Diagnosis**

**1- Gram stain** ( G<sup>+</sup> cocci, grape , tetrad , pairs and singles some time in the form of short chains at least 5 cells ) .

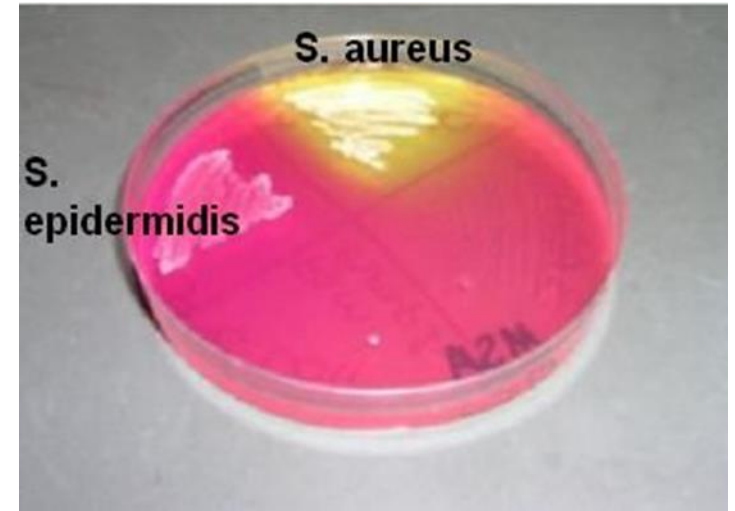
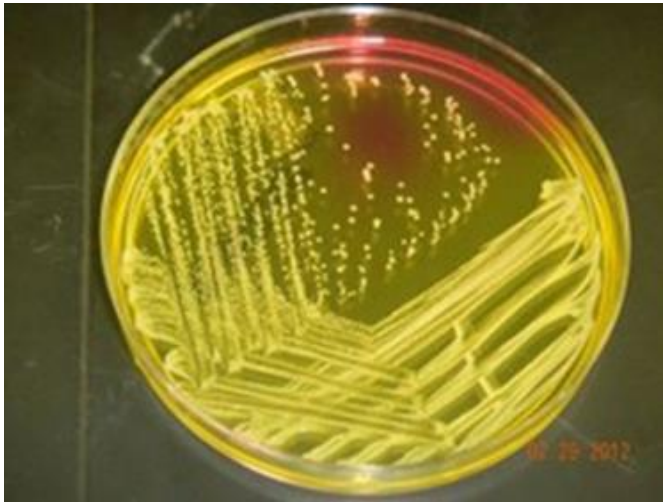
**2- Blood agar (Enriched media):**  $\beta$  hemolytic (complete hemolysis) in *S. aureus* only.

**3- Mannitol salt agar (differential media )** it contains mannitol 7.5 % sodium chloride and phenol red indicator. NaCl inhibits organisms other than *Staphylococci* if the mannitol is fermented to produce acid the phenol red in the medium changes color from red to yellow.

**4- Staphylococcus medium (110):** (Selective media)

It contains NaCl and mannitol, but it lacks phenol red, no color change and takes place as mannitol is fermented.







**5- DNA ase agar :** Contain (Mannitol, DNA, Bromothymol blue as indicator, it's specific for *S.aureus* (Coagulase + ve) that able to hydrolyze DNA.

pH Natural       $\longrightarrow$       Acid  
( blue )                      ( yellow )

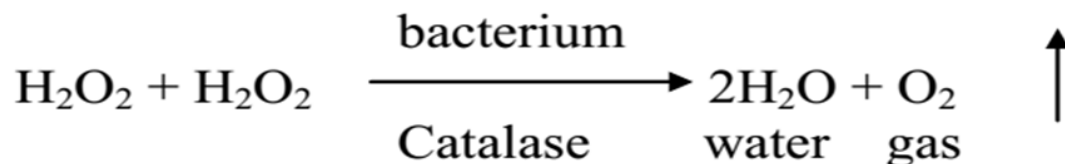
**6- Catalase test:-** To test for present of enzyme Catalase to differentiate between genera.

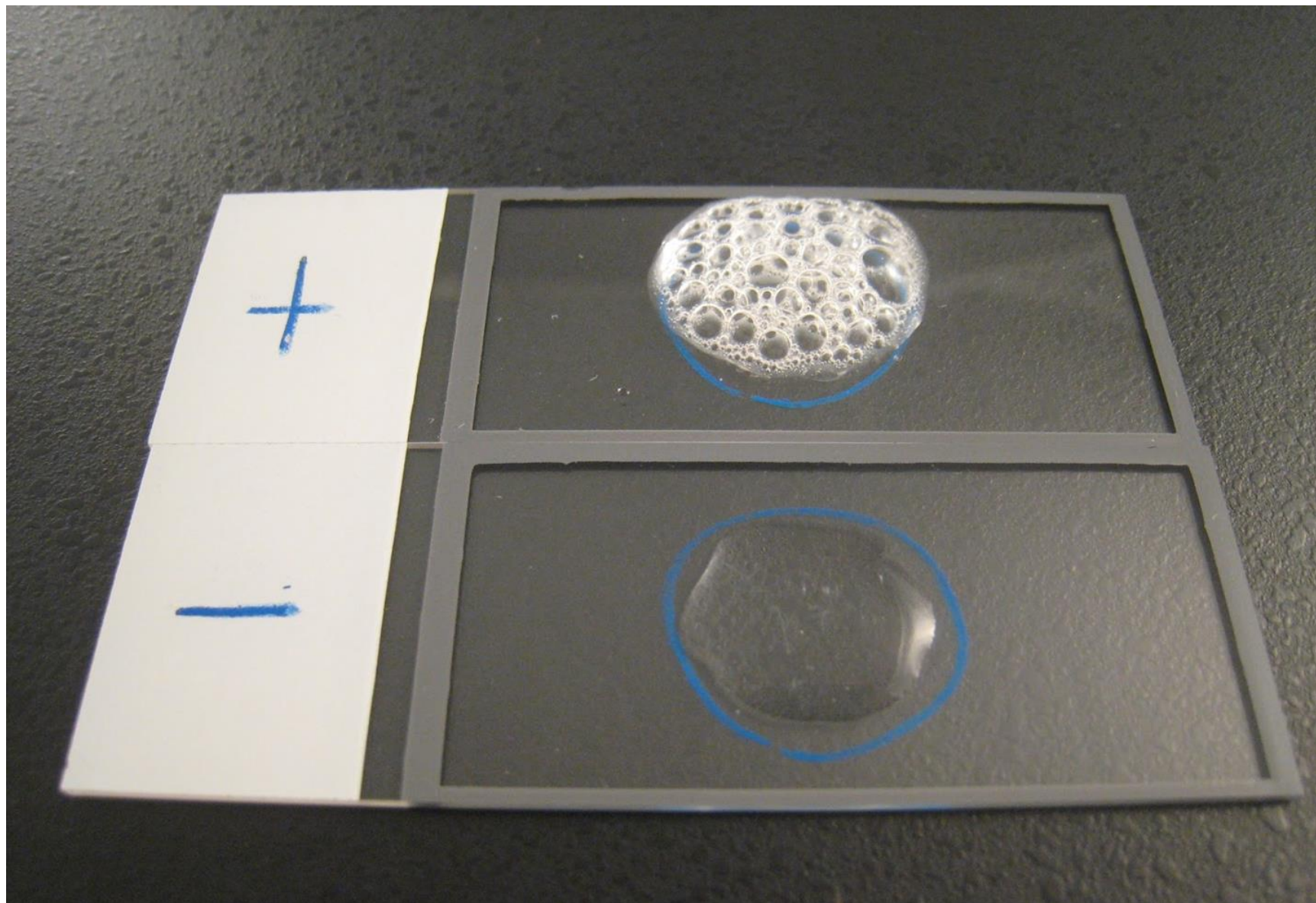
**Method:-**

A drop of hydrogen peroxide solution (3 % hydrogen peroxide –  $H_2O_2$ ) is placed on slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (The release of oxygen) indicates a positive test.

**Result:-** Positive test immediate bubbling by ( $O_2$ ) formed or gas Liberation.

**Mechanism:**





**7- Coagulase test:** The test is an excellent definitive test for confirming identification of *S.aureus* (detect clumping factor present in *S.aureus* and absent from most Staphylococci).

The Two method for identification Staph-coagulase as positive

**A) Slide coagulase test:-** This test detects clumping factor (bound coagulase) which is cell wall component that causes the organisms to clump when mixed with plasma.

- Emulsify a Staphylococcal colony in a drop of water on a microscope slide with a minimum of spreading. If the isolate does not form a smooth, milky suspension, does not proceed. Add large drop of plasma ( rabbit or human plasma) and mix well .

\* Positive result agglutination of suspension during 10sec.

**B) Tube coagulase test:-** This test detects an extracellular enzyme called coagulase. It activates a coagulase reacting factor (CRF) normally present in plasma, causing the plasma to clot by conversion of fibrinogen to fibrin.

- In sterile glass tube add ( 0.5 ml ) of undiluted plasma (human or rabbit plasma).

- Add 0.5 ml of an ( 18- 24 hr . ) pure broth of *Staphylococcus* .

- Rotate tube gently and incubation at 37 C ° for 4 hr .

- observe every 30 min for a clot forming .

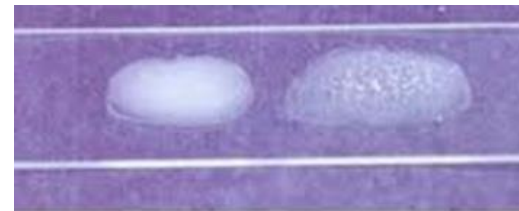
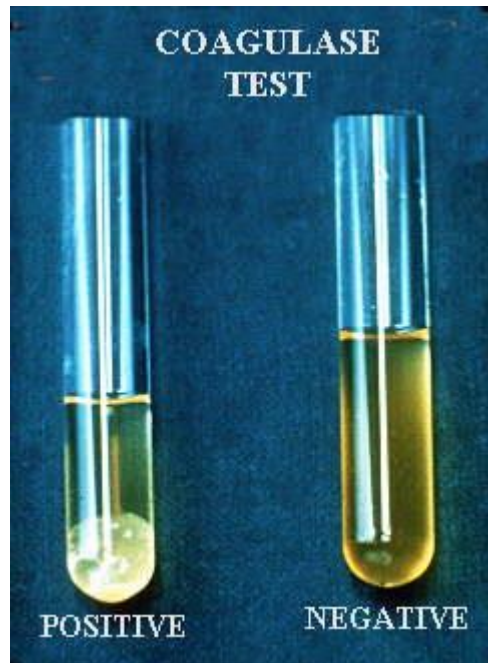


Table (1): *Stphaylococcal sp.* tests

Staphylococcal sp.	Coagulase	Hemolysin	Pigmentation	Mannitol fermentation	DNA ase
<i>S. aureus</i>	+	+	+	+	+
<i>S. epidermidis</i>	-	-	+/-	-	-
<i>S. sparophyticus</i>	-	-	-	+/-	-