

## Lab1: The Staphylococci

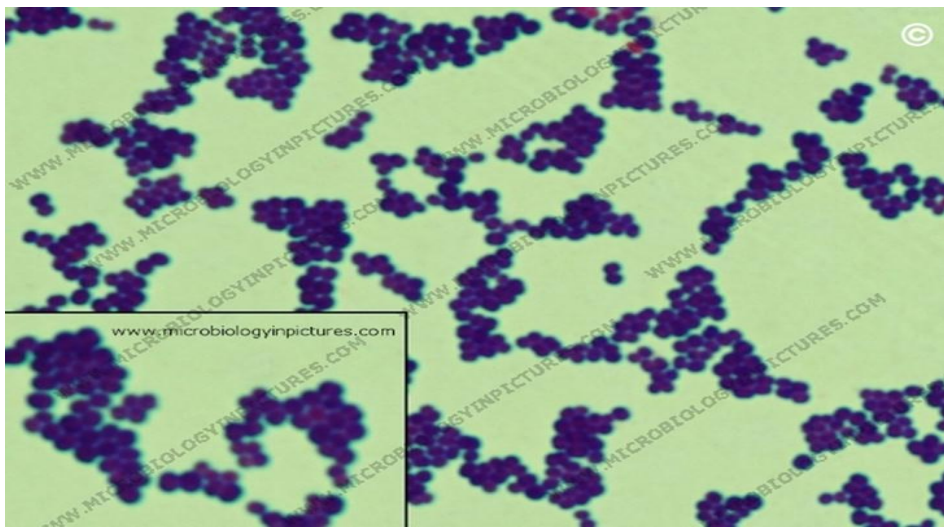
The staphylococci are gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal microbiota of the skin and mucous membranes of humans.

The genus *Staphylococcus* has at least 40 species. The three main species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*.

*Staphylococcus aureus* is **coagulase-positive**, which differentiates it from the other species.

### Characteristics Of *Staphylococcus aureus* Bacteria

- ❖ **gram-positive cocci in clusters**
- ❖ **non-motile**
- ❖ **non-spore-forming**
- ❖ **catalase: positive**
- ❖ **oxidase: negative**
- ❖ **facultative anaerobic**



*Staphylococcus aureus* gram stain

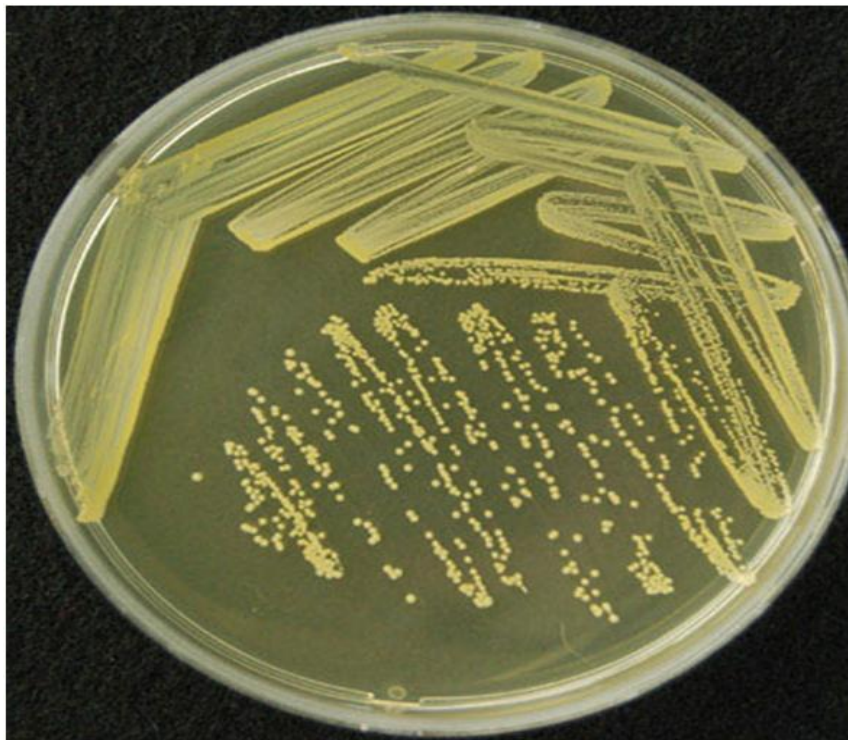
**Culture:**

Media used-:

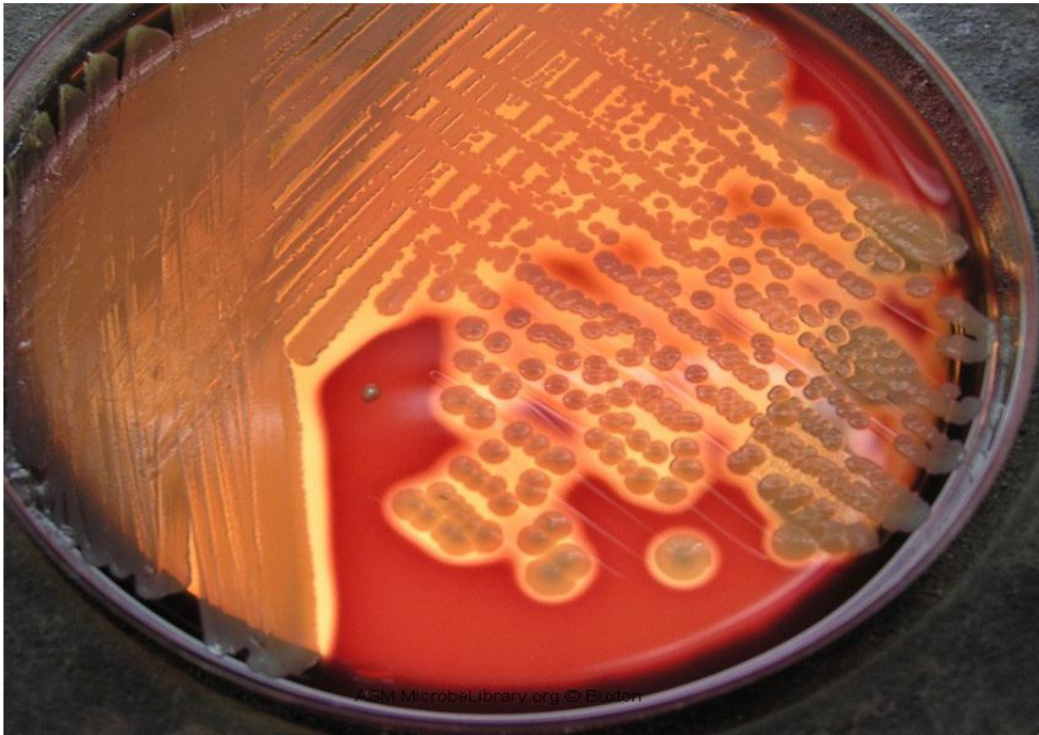
- ❖ Non selective media: Nutrient agar, Blood agar, MacConkey agar, Brain heart agar .
- ❖ Selective media: Mannitol Salt Agar ,Staphylococcus Medium No. 110.

**Cultural Characteristics:**

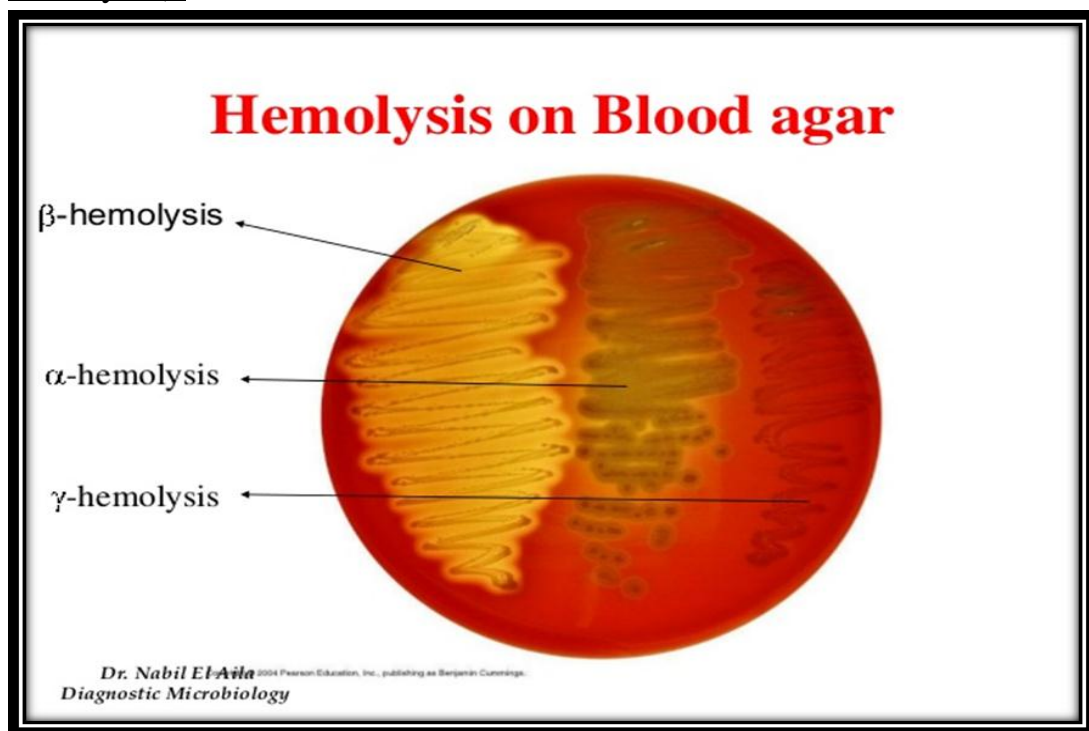
- ❖ **On nutrient agar:** The colonies are large, circular, convex, smooth, shiny and opaque. Most strains produce golden yellow pigments.

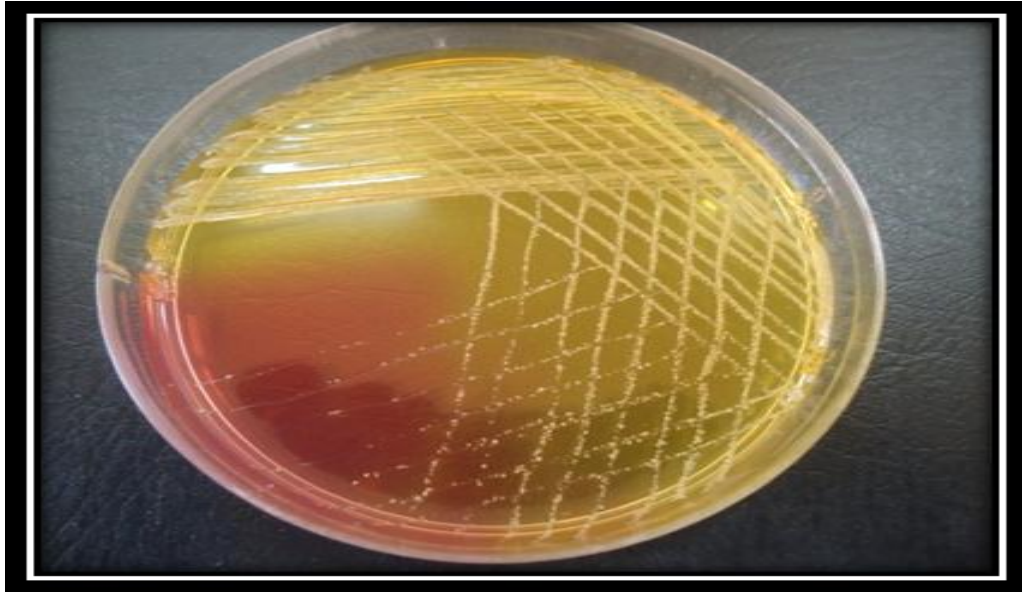
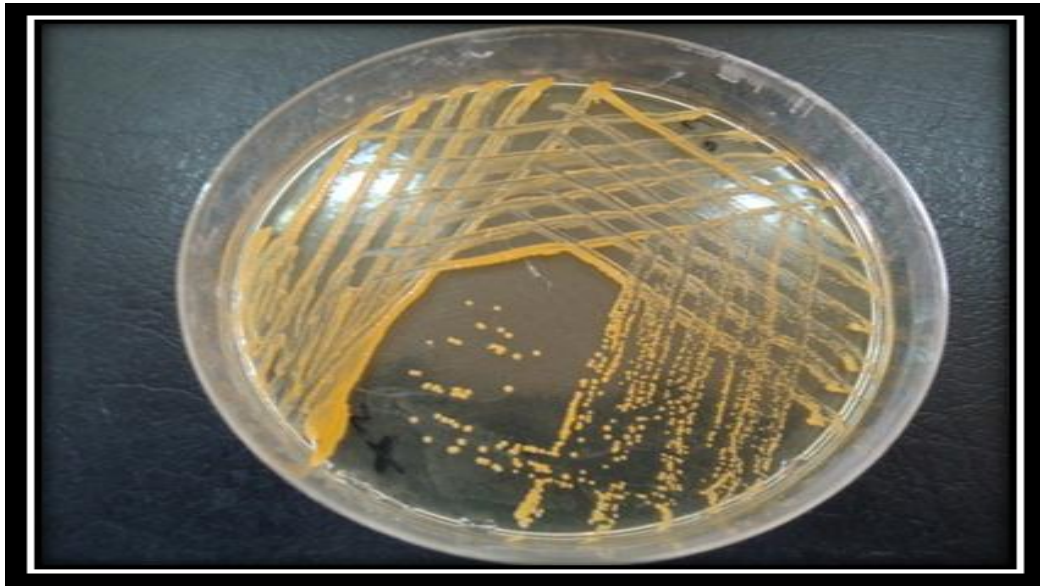


- ❖ On MacConkey's agar- The colonies are small & pink in colour.
- ❖ On blood agar- Most strains produce  $\beta$ - haemolytic colonies.



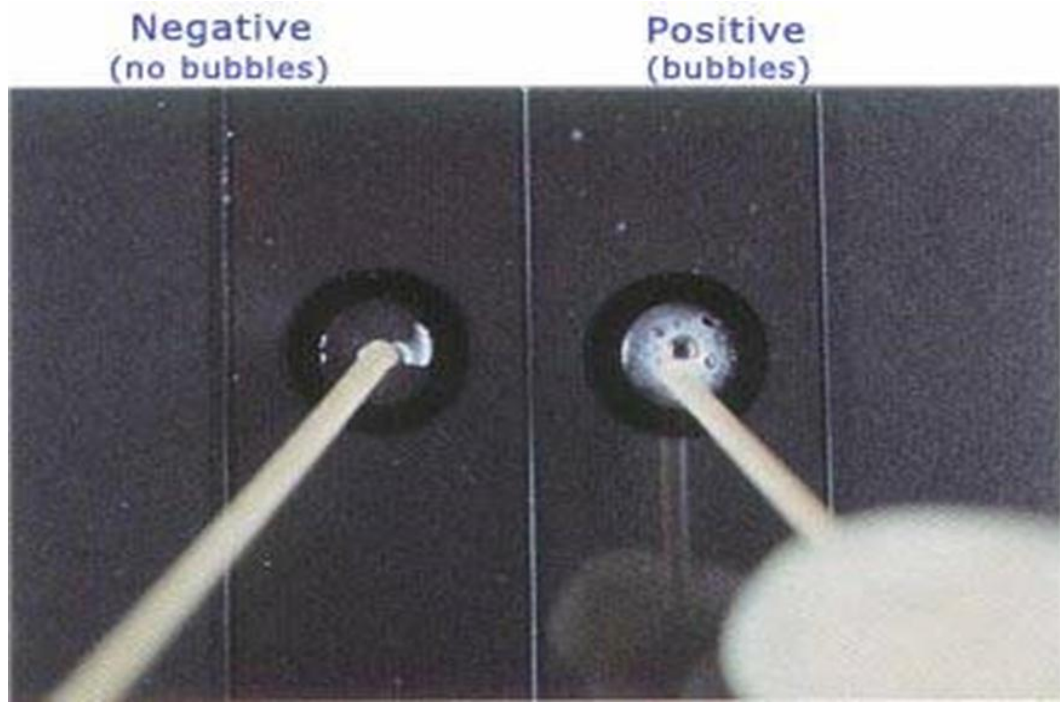
*S. aureus* appears golden-yellow colonies, often with beta hemolysis, when grown on blood agar plates. **Beta hemolysis ( $\beta$ -hemolysis)**, sometimes called **complete hemolysis**, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. There are three *Staphylococcus aureus* hemolysis (alpha-hemolysin, beta-hemolysin and gamma-hemolysin).



❖ On Mannitol Salt Agar,❖ On Staphylococcus Medium No. 110.**Biochemical tests**

1. **Catalase test** : catalase is an enzyme that removes hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by catalyzing the breakdown of the molecules into water and oxygen.

- ✚ The enzyme is produced by all species of *Staphylococcus*, so they can be differentiated from *Streptococci*, which cannot produce the enzyme.
- ✚ Procedure. Take a colony of the organism to be tested and place on a glass slide
- ✚ Add a drop of 3% hydrogen peroxide onto the colony.
- ✚ The immediate formation of oxygen bubbles is evidence that the organism produces catalase (positive reaction).
- ✚ Few or no bubbles after 20 seconds indicates a negative reaction.



## 2-Coagulase test-

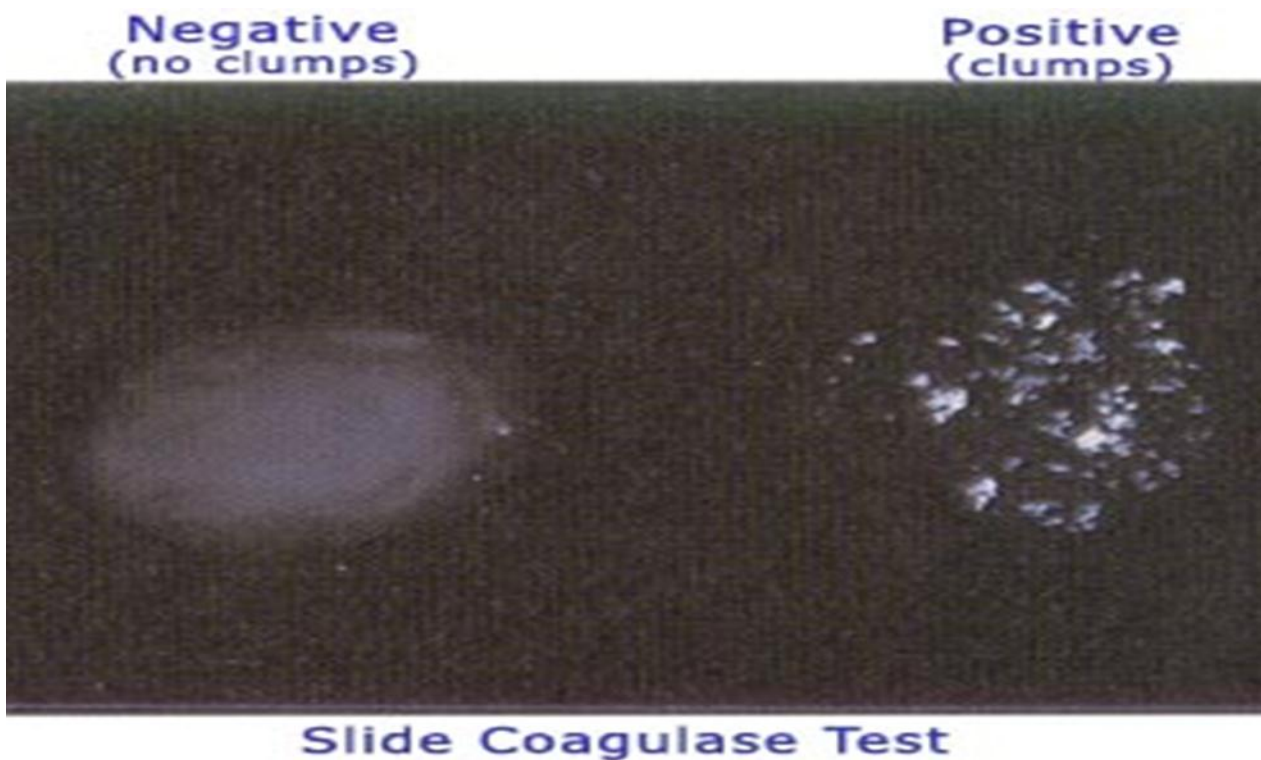
- **Slide coagulase test- Positive.**
- **Tube coagulase test- Positive**

**2-Coagulase test:** this test is used to differentiate Staphylococcus aureus (positive) from Coagulase Negative Staphylococcus (CONS).

**Coagulase** is an enzyme produced by *staphylococcus aureus*, which converts (soluble ) fibrinogen in plasma to (insoluble) fibrin.

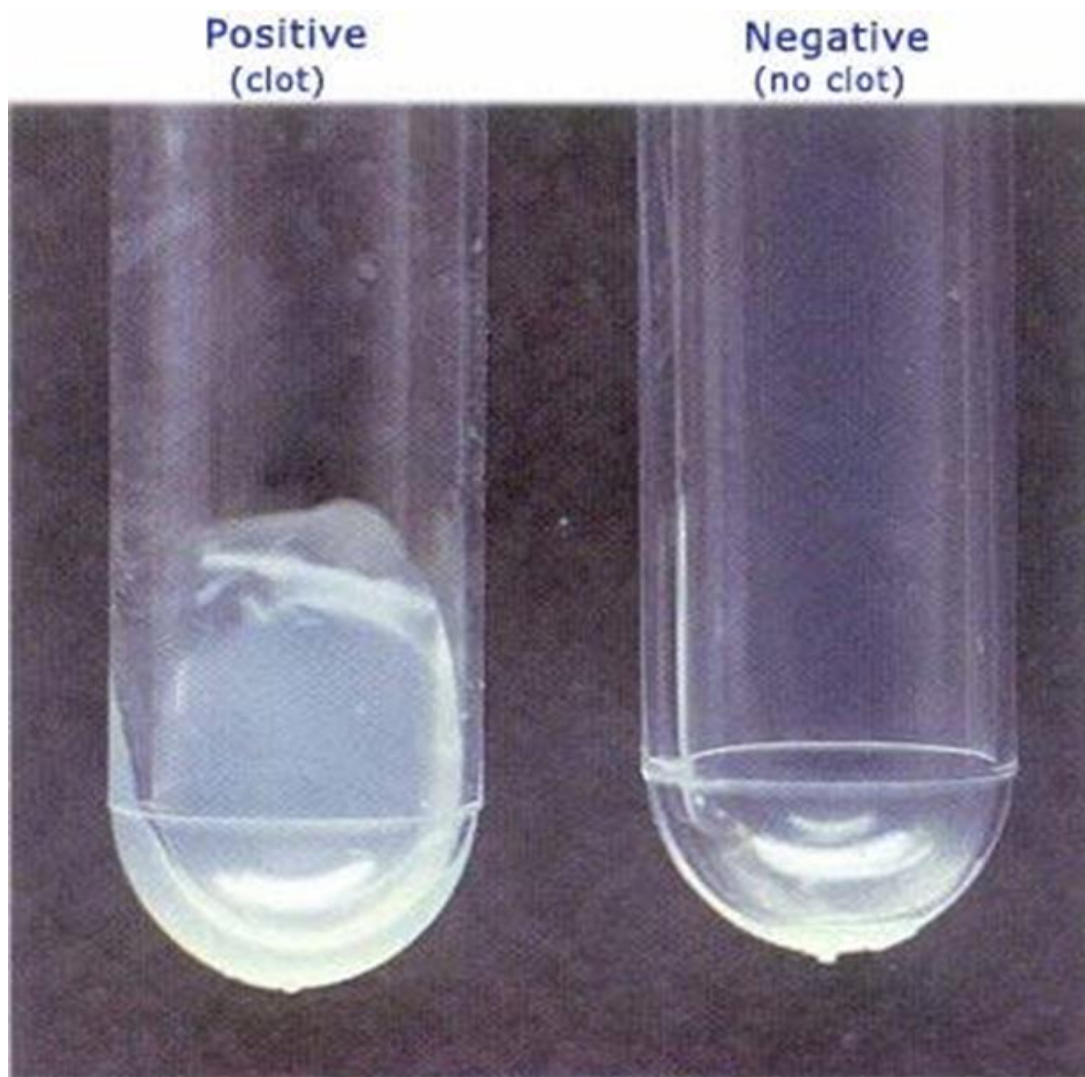
**1-Slide coagulase test** is done to detect bound coagulase or clumping factor and clumping factor is a protein located on the surface of *Staphylococcus aureus* cells

- **Procedure.** Place a drop of sterile water on a slide, then emulsify a colony to be tested.
- **Add a drop of rabbit plasma, and shake the slide gently for 5 to 10 seconds and look for clumping (coagulation)**



2-Tube coagulase test is done to detect free coagulase. The test detects staphylocoagulase enzyme, which is also known as free coagulase.

- Procedure. Emulsify several isolated colonies of test organism in 0.5 ml of rabbit plasma to give a milky suspension.
- Incubate tube at 35°C for 4 hours.
- The tube should be checked every 30 minutes for up to 4 hours to see if a clot has formed.
- Formation of a clot indicates a positive reaction.



Tube Coagulase Test

**Virulence Factors of *S. aureus* :**

These include

**1- Cell associated factors**

a) cell associated polymers

1. Cell wall polysaccharide
2. Teichoic acid
3. Capsular polysaccharide

b) cell surface proteins:

1. Protein A
2. Clumping factor (bound coagulase)

**2- Extracellular factors**

1. Enzymes: such as Catalase, Lipase, DNAase and Staphylokinase
2. Toxins: such as Cytolytic toxins, Enterotoxin,

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