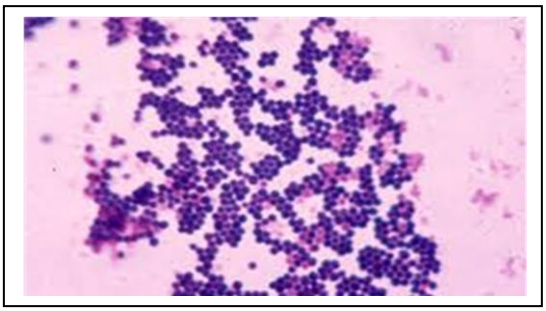
Staphylococci

***Staphylococci* is** a genus of Gram-positive bacteria in the family *Staphylococcaceae* from the order *Bacillales*. Under the microscope, they appear spherical (cocci), and form in grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms (capable of growth both aerobically and anaerobically).

Laboratory important species:



1)*Staphylococcus aureus.*

2)Staphylococcus saprophyticus.

3)Staphylococcus eipdermidis.

Shape and pigmentation:

- *Staphylococcus* spherical bacteria with diameters (1.5 - 0.8 um), positive for Gram stain.
- They are organized in the form of clusters similar to grape clusters, as they show the ability to divide in more than one level, while their members remain linked to each other when planted on solid media, but they can appear in liquid media, single or double, or in the form of very short chains of no more than four cells.
- The germ is non-motile.

Bacterial properties:

Bacteria are facultative aerobic or anaerobic. The optimum temperature for their growth is 37 C, but they grow at a temperature between 10-43 °C. The optimum pH for their growth is between (7.4-7.6).

- **Coagulase Test:**

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which do not produce coagulase. i.e Coagulase Negative *Staphylococcus* (CONS).

Principle of Coagulase Test:

Coagulase is an enzyme-like protein and causes plasma to clot by converting fibrinogen to fibrin. *Staphylococcus aureus* produces two forms of coagulase: bound and free.

- Bound coagulase (clumping factor) is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alternation of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. This doesn't require coagulase-reacting factor.
- Free coagulase involves the activation of plasma coagulase-reacting factor (CRP), which is a modified or derived thrombin molecule, to from a coagulase-CRP complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

Procedure and Types of Coagulase Test:

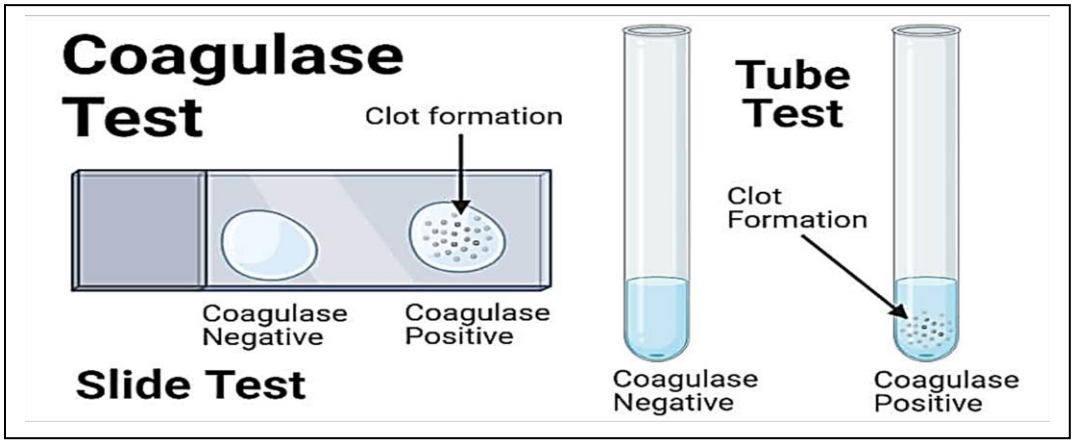
I. Slide Test (to detect bound coagulase):

1) Place a drop of physiological saline on each end of a slide, or on two separate slides.
2) With the loop, straight wire or wooden stick, emulsify a portion of the isolated colony in each drops to make two thick suspensions.
3) Add a drop of human plasma to one of the suspensions, and mix gently.
4) Look for clumping of the organisms within 10 seconds.
5) No plasma is added to the second suspension (control) to differentiate any granular appearance of the organism from true coagulase clumping.

II. Tube Test (to detect free coagulase):

1) Dilute the plasma 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline).
2) Take 3 small test tubes and label as T (Test), P (Positive Control) and N (Negative Control). Test is 18-24 hr. broth culture; Positive control is 18-24 hr. S. aureus broth culture and Negative control is sterile broth.
3) Pipette 0.5 ml of the diluted plasma into each tube.

4) Add 5 drops (0.1 ml) of the Test organisms to the tube labelled "T", 5 drops of S. aureus culture to the tube labelled "P" and 5 drops of sterile broth to the tube labelled "N".
5) After mixing, incubate the three tubes at 35-37 °C.
6) Examine for clotting after 1 hours. If no clotting has occurred, examine at 30 minutes intervals for up to 6 hours



- **Catalase Test:**

This test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produces an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. Normally 3% H_2O_2 is used for the routine culture while 15% H2O2 is used for detection of catalase in anaerobes.

Principle of Catalase Test:

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

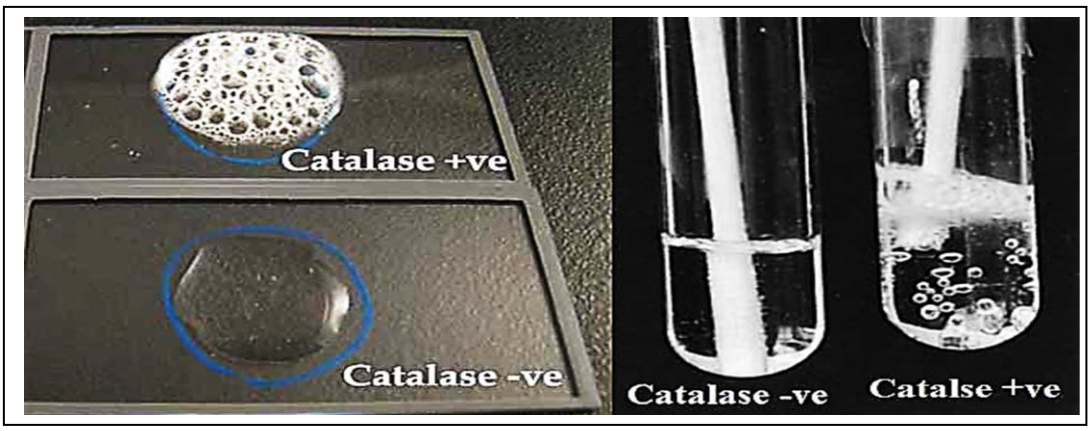
Purpose or Uses of Catalase Test:

- The morphologically similar *Enterococcus* or *Streptococcus* (catalase negative) and *Staphylococcus* (catalase positive) can be differentiated using the catalase test.
- Also valuable in differentiating aerobic and obligate anaerobic bacteria.
- Semiquantitative catalase test is used for the identification of *Mycobacterium tuberculosis*.
- It is used to differentiate aerotolerant strains of *Clostridium*, which are catalase negative, from *Bacillus* species, which are positive.
- Catalase test can be used as an aid to the identification of *Enterobacteriaceae*.

Procedure of Catalase Test

I. Tube Method

1) Pour 1-2 ml of hydrogen peroxide solution into a test tube.
2) Using a sterile wooden stick or a glass rod, take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution.
3) Observe for immediate bubbling.

II. Slide Method

1) Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2) Place a drop of 3% H2O2 in the glass slide.
3) Observe for the evolution of oxygen bubbles.