Lab 10 (Biochemical Tests)

Biochemical Tests:

- Biochemical reactions are very important in the identification of bacterial isolates and in the identification of different bacterial species.
- These tests depend on the presence of certain enzymes, such as catalase, oxidase, urease, gelatinase, etc., produced by the bacteria.
- Different bacteria produce varying spectra of enzymes. For example, some enzymes are necessary for the bacterium's individual metabolism, and some facilitate the bacterium's ability to compete with other bacteria or establish an infection.
- Tests that measure single bacterial enzymes are simple, rapid, and generally easy to interpret.
- They can be performed on organisms already grown in culture and often provide presumptive identification.

Catalase Test:

- To differentiate between two genera staphylococcus and streptococcus .
- Some bacteria contain flavoproteins that reduce O_2 resulting in the production of hydrogen peroxide (H_2O_2) or superoxide (O_2). These are toxic for obligate aerobes and facultative anaerobes.
- Many bacteria produce enzymes to protect them self against superoxide (O_2), these enzymes are catalase, peroxidase or superoxide dismutase , which catalyze the destruction of H_2O_2 or O_2 as follows :

$$2O_2 + 2H+ \xrightarrow[\text{Dismutase}]{\text{Superoxide}} O_2 + H2O2$$

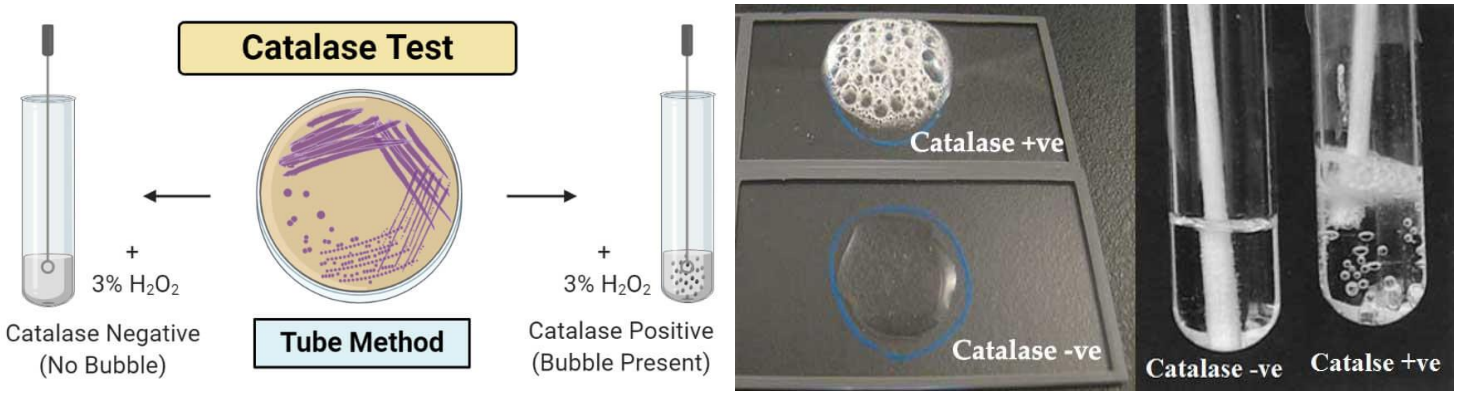
$$2H_2O_2 \xrightarrow[\text{Peroxidase}]{\text{Catalase or}} 2\,H_2O + O_2$$

- Catalase production can be noted by mixing H_2O_2 with the tested bacteria. Bubbles of O_2 represent a positive catalase test, and the absence of bubbles represent a negative catalase test.
- Most strict anaerobes lack both enzymes and cannot tolerate O_2
- Staphylococci and Micrococci are catalase positive (bubble formation) while Streptococcus and Enterococcus spp. are catalase-negative (no bubble formation).

Procedure

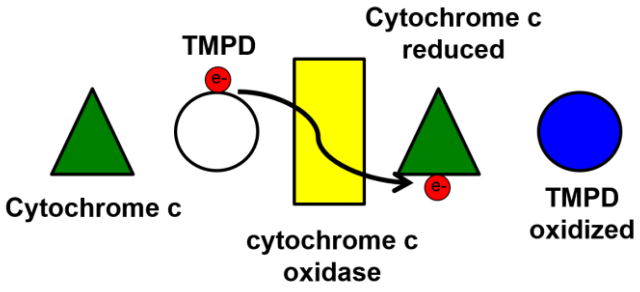
1. Label two nutrient broths or nutrient agar slants.
2. Using aseptic technique, inoculate one agar slant or broth with staphylococcus and the other tube with streptococcus.

3. Incubate all the cultures at 37ºC for 18 to 24 hours.
4. Add few drops of 3% H_2O_2 over the growth of the slants or broths and observe the appearance of gas bubbles.
5. Record the results.



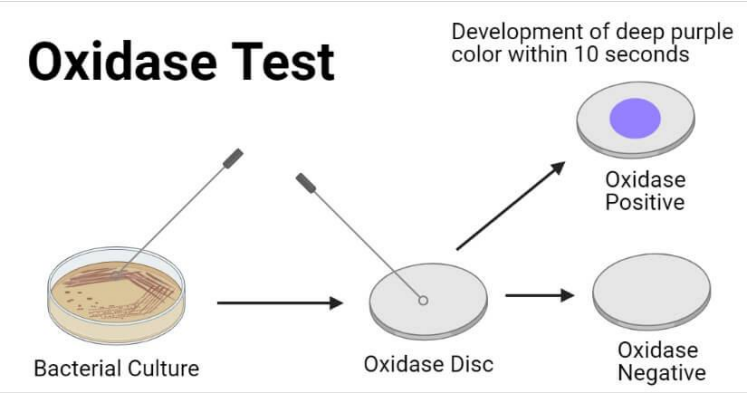
Oxidase Test:

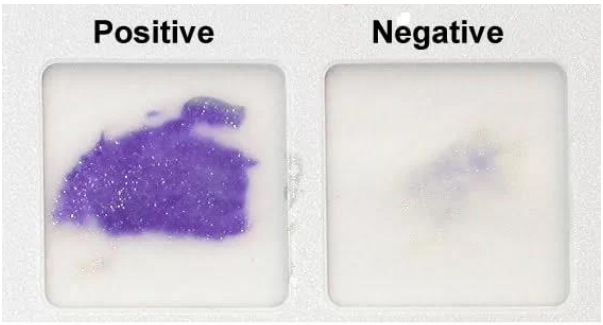
- Oxidase enzymes have an important role in the electron transport system during aerobic respiration.
- This test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain.
- All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration.
- **This test is used in the differentiation between the oxidase positive (purple color formation) bacteria (*Neisseria*, *Pseudomonas* and *Vibrio*) and the other oxidase negative (no color change) gram-negative bacteria particularly Enterobacteriaceae such as *E. coli.***
- The oxidase reagent that used is tetramethyl-p-phenylenediamine dihydrochloride (TMPD).
- The test principle is simplified as follows:



Main Procedure method

1. Transfer bacterial growth to filter paper.
2. Add few drops of oxidase reagent on the bacterial growth.
3. Observe the color change after 20 to 30 seconds.





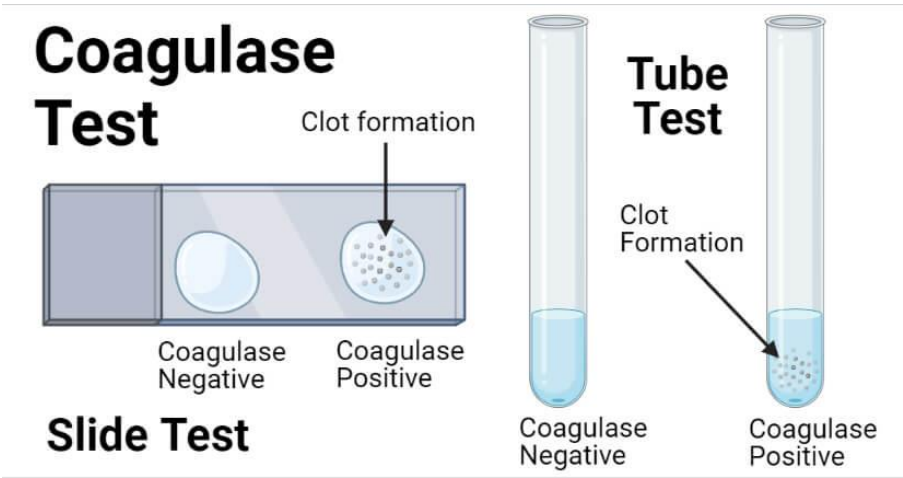
Oxidase Test

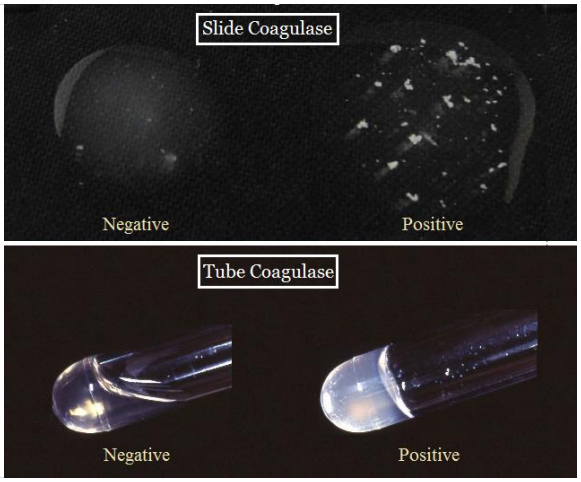
Coagulase Activity Test:

- The coagulase test is used to differentiate pathogenic *Staphylococcus aureus* from other non-pathogenic coagulase negative staphylococcus spp.
- Coagulase is enzyme produced by **pathogenic Staphylococci** that clot blood plasma.
- Citrate is usually added to act as anticoagulant and prevent false positive results, however; *S. aureus* strains are usually capable of coagulating EDTA-treated plasma in the tube test and will produce clumps of cells in the slide test.

Procedure

1. Add 0.5 ml of citrated rabbit plasma to two small test tubes, label the tubes with name of bacteria.
2. Add 0.5 ml broth culture of ***S. aureus*** to one tube and 0.5 ml broth culture of *S. epidermis* to the other tube.
3. Incubate the broth cultures at 37ºc for 1 to 4 hours in water bath.
4. Examine the broth cultures for the presence of clots (coagulase positive staph. aureus) or absence of clots (coagulase negative).
5. Record the results.



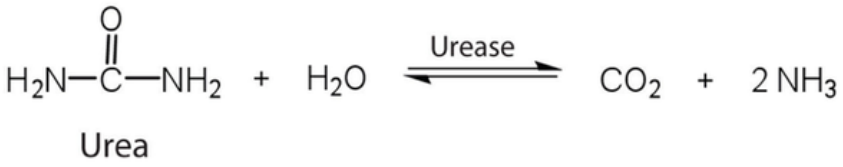


Coagulase positive (clot formation): *Staphylococcus aureus*

Coagulase negative (absence of clot): *Staphylococcus epidermis*

Urease Test:

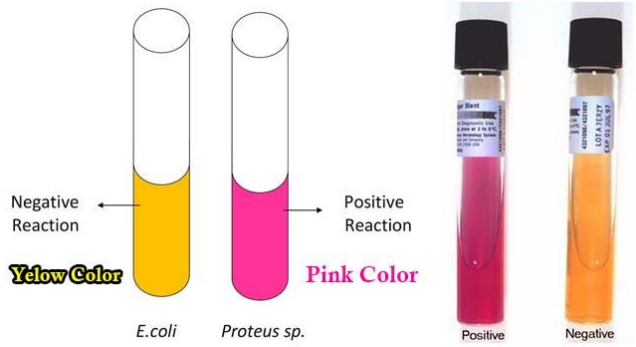
- Some bacteria such as Proteus, Klebsiella, Pseudomonas are able to produce an enzyme called urease that hydrolyses urea, forming the end products ammonia, CO_2 and water as follows:



- This test is done to detect the urease production, by growing bacteria in urea agar or broth containing urea and a pH indicator such as phenol red.
- After hydrolysis of urea, ammonia accumulates in medium and makes it alkaline.
- The increase in PH causes the indicator to change into deep pink (positive test).
- No. change in color indicates negative test.

Procedure

1. Label two urea agar or broth with the name of the bacterium to be tested.
2. Using aseptic technique, inoculate one medium with the bacterium Proteus and the other medium with the bacterium *E. coli*.
3. Incubate at 37ºC for 4 hours or more.
4. Examine change in color and record the results.



- Positive result (pink color): *Proteus sp.*

- Negative result (yellow color): *Escherichia coli.*

Gelatinase Hydrolysis (Gelatin Liquefaction) Test

- Certain bacteria such as Staphylococcus sp. and Enterobacteriaceae are able to hydrolyze gelatin by secreting a proteolytic enzyme (gelatinase).
- Gelatinase is important in pathogenic bacteria to dissolve collagen in connective tissues.
- The resulting amino acids can be used as nutrients by the bacteria.
- Gelatin liquefaction can be tested by stabbing nutrient gelatin.
- Following incubation, the cultures are placed in a refrigerator for about 1/2 to 1 hours.
- If gelatin has been hydrolyzed, the medium will remain liquid, and if gelatin has not been hydrolyzed, the medium will re-solidify after refrigeration.

Procedure

1. Label two nutrient gelatin tubes.
2. Using aseptic technique, inoculate (stab) one of the tube with *S. aureus* or *P. vulgaris* and the other tube with *E .coli*.
3. Incubate the tubes for 24 hours or more at 37ºC.
4. Read and record the results after refrigeration.

- Positive result / left (gelatin liquification): *Staphylococcus aureus*

- Negative result / right (gelatin remaining solid): *Escherichia coli.*