Biochemical Tests of Bacteria

Part one

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Biochemical tests are important in the identification of bacterial species.

These tests depend on the presence of certain enzymes. Different bacteria produce different enzymes such as catalase, oxidase, urease, gelatinase, etc., produced by the bacteria.

- Catalase Test
- Coagulase Test
- Optochin sensitivity testing
- Bacitracin sensitivity testing
- CAMP Test
- Bile Esculin Test
- Starch hydrolysis test
- Gelatinase Test

Catalase Test

To determine the ability of bacteria that produces Catalase enzyme which degrades the hydrogen peroxide.

İn aerobic organisms, during aerobic respiration, oxygen serves as hydrogen acceptor and hydrogen peroxide is formed in the cell. High concentration of H2O2 is formed which is toxic to cell. Bacteria posses the catalase enzyme converts hydrogen peroxide into oxygen and water.

Procedure:

Catalase production can be determined by addition of the substrate H_2O_2 on bacterial culture.

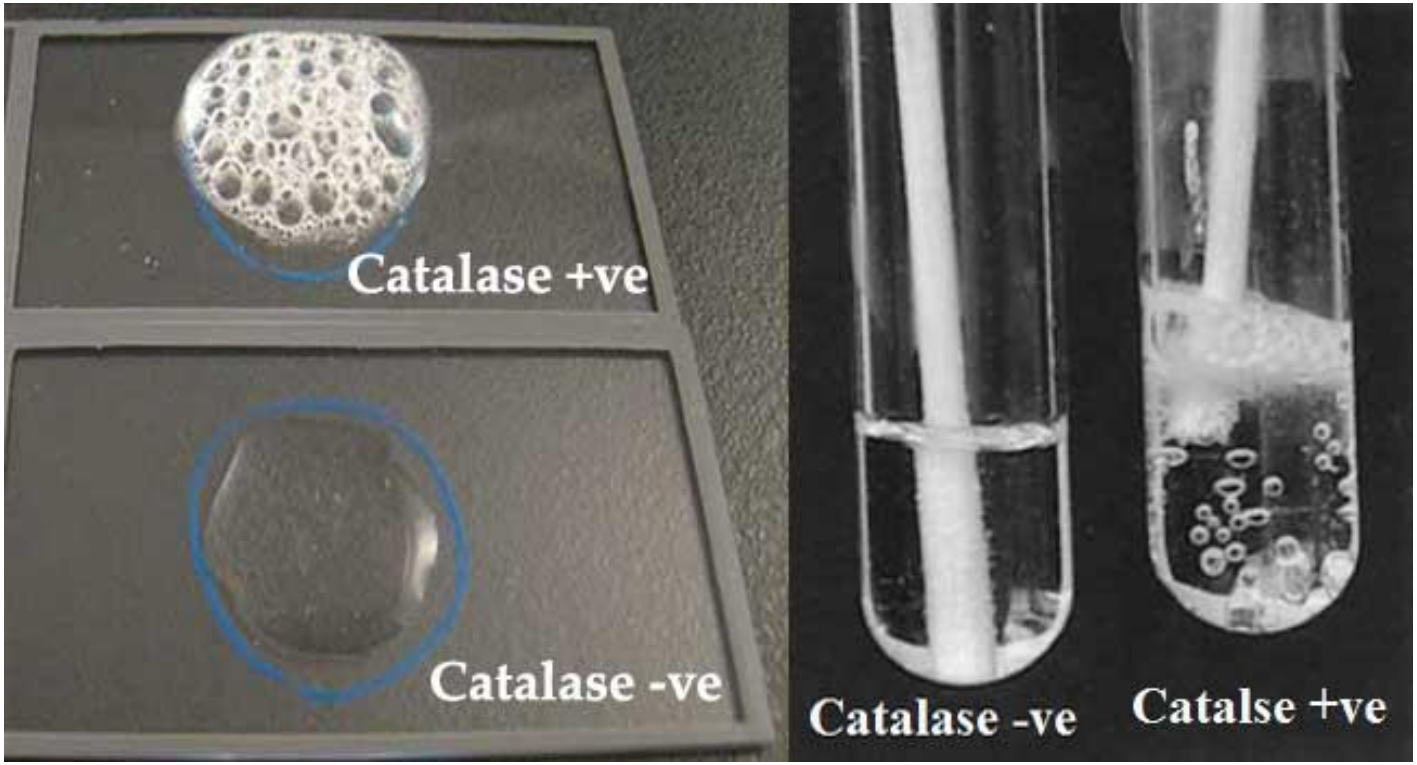
1. Slide Method:

- Pure growth of the organisms will transfer to the clean slide by using inoculation loop or glass rod.
- Immediately add a drop of 3% hydrogen peroxide on bacterial culture.
- Observe the bubble formation.

2. Tube Test:

- Take one ml of 3 % hydrogen peroxide in test tube.
- Small amount of bacterial culture introduce into the solution
- Immediately observe the effervescence.

Result Interpretation of Catalase Test and Examples



Positive: Copious bubbles produced, active bubbling

Examples: *Staphylococcus* spp. and the *Micrococcus* spp.

Negative: No or very few bubbles produced.
Examples: *Streptococcus and Enterococcus* spp

Coagulase Test

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in tube or agglutinate cocci in slide. This test is useful in differentiating ***S.aureus*** from other **coagulase-negative staphylococci.**

Most strains of ***S.aureus*** produce two types of coagulase, **free coagulase** and **bound coagulase.** While free coagulase is an enzyme that is secreted extracellularly, bound coagulase is a cell wall associated protein.

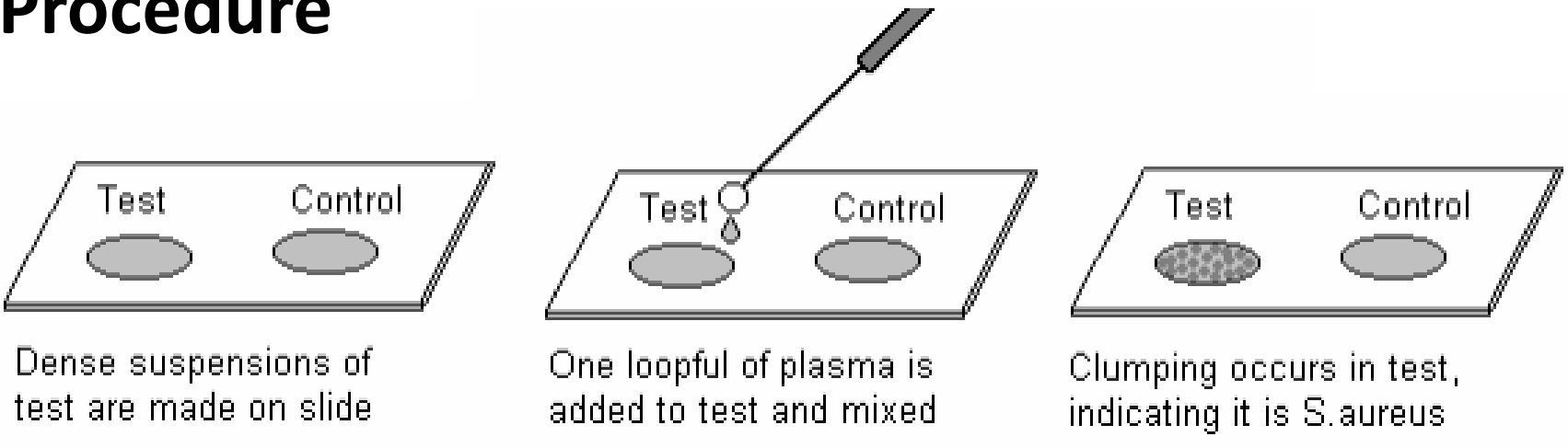
Free coagulase is detected in tube coagulase test and bound coagulase is detected in slide coagulase test. Slide coagulase test may be used to screen isolates of *S. aureus* and tube coagulase may be used for confirmation..

SLIDE COAGULASE TEST:

Principle: The bound coagulase is also known as **clumping factor**.

It cross-links the α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the bacterial cell wall. As a result, individual coccus stick to each other and clumping is observed

Procedure



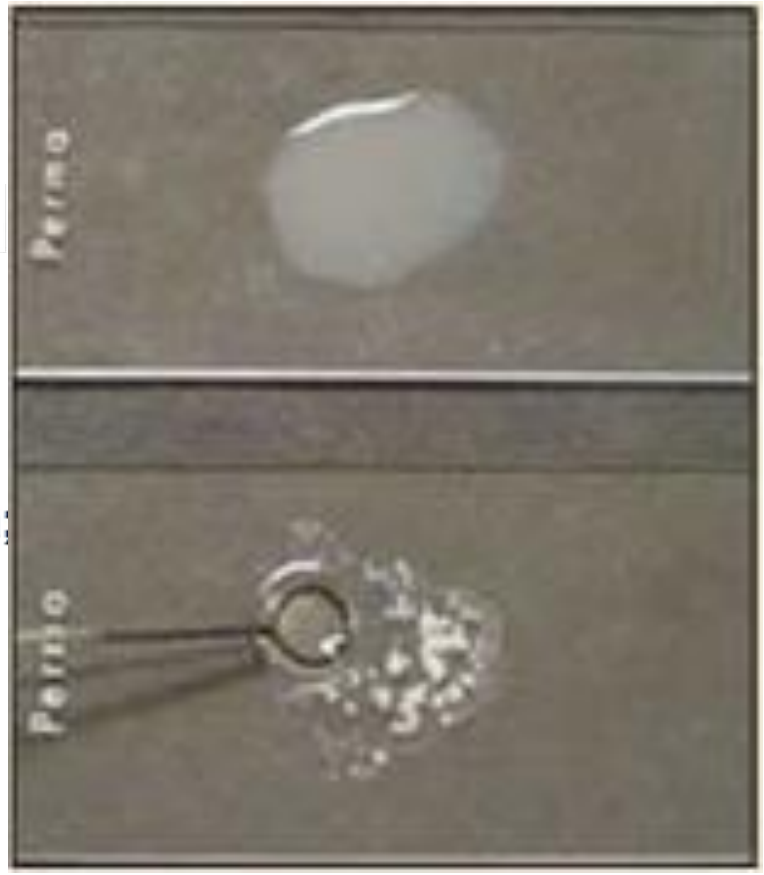
Interpretation result

Negative – smooth suspension

Negative – Staphylococcus epidermidis

Positive – white fibrin clots in plasma

Positive – Staphylococcus aureus



TUBE COAGULASE TEST:

Principle: The free coagulase secreted by ***S. aureus*** reacts with coagulase reacting factor (CRF) in plasma to form a coagulase-CRF complex. The complex then reacts with fibrinogen to form the fibrin clot in a test tube

Procedure

5 ml of the diluted plasma (Add 0.2 ml plasma in 1.8 ml is added to saline)added to a test tube. About 5 drops of the test organism culture are added to the test tube.

The test tube is mixed and incubated at 37°C for an hour.
The tube is finally observed for the clot formation. If no clotting is observed, the tube should be examined at 30 minutes interval of up to 6 hours.

Interpretation result

Positive – formation of fibrin clot;

Positive – Staphylococcus aureus

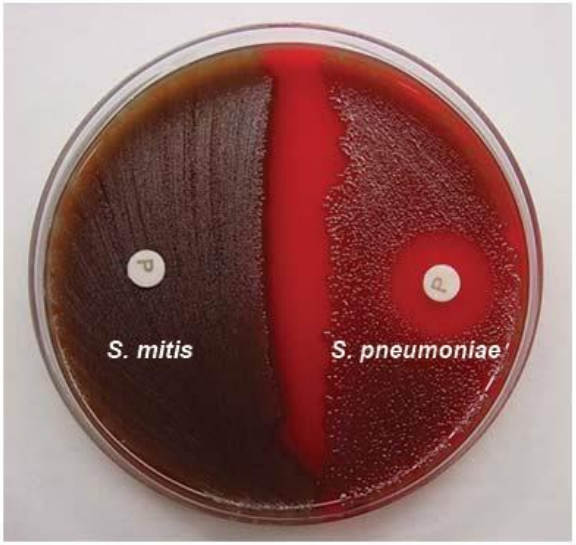
Negative – no clot is formed

Negative – Staphylococcus epidermidis

Optochin sensitivity testing

This is a differential test used to distinguish between organisms sensitive to the antibiotic optochin and those not. **Optochin** is an antibiotic that interferes with the ATPase and production of adenosine triphosphate (ATP) in microorganisms.

This test is used to distinguish ***Streptococcus pneumoniae*** (optochin sensitive (pictured on the right below)) from other α-hemolytic streptococci (optochin resistant (***Streptococcus mitis*** is pictured on the left below).



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

Bacitracin sensitivity testing

Principle

This is a differential test used to distinguish between organisms sensitive to the antibiotic bacitracin and those not. Bacitracin is a peptide antibiotic produced by *Bacillus subtilis*

The antibiotic bacitracin inhibits the synthesis of bacterial cell walls by interfering the peptidoglycan synthesis of bacteria.

A disk saturated with a small amount of bacitracin (0.04 units) is placed on an agar plate, allowing the antibiotic to diffuse into the medium and inhibit the growth of susceptible organisms. After incubation, the inoculated plates are examined for zones of inhibition surrounding the disks.

If the organism grows up to the edge of the disk, it is resistant to the antimicrobial compound infusing the disk. If there is a zone around the edge of the disk where the organism has not grown, the organism is susceptible to the antimicrobial in the disk.

Bacitracin sensitivity test is commonly used to distinguish between the β-hemolytic streptococci: ***Streptococcus agalactiae*** (bacitracin resistant) and ***Streptococcus pyogenes*** (bacitracin sensitive).

Starch hydrolysis test (amylase production test)

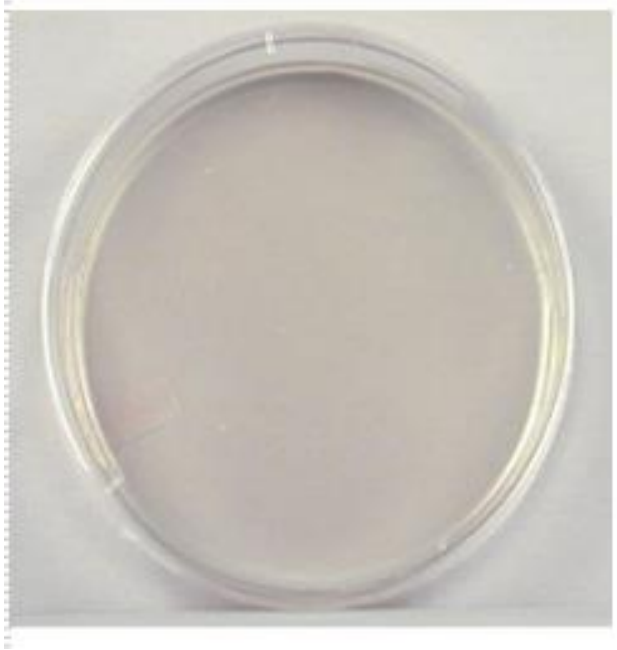
This test is used to identify bacteria that can hydrolyze starch (**amylose** and **amylopectin**) using the enzymes **α-amylase and oligo-1,6-glucosidase.**

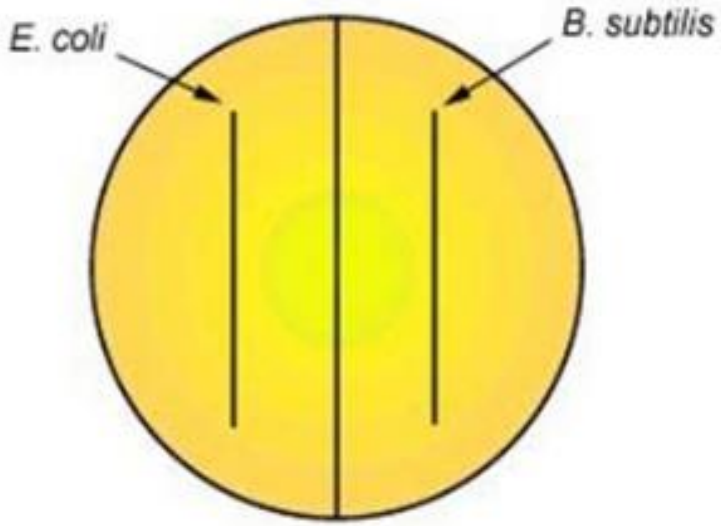
Because of the large size of amylose and amylopectin molecules, these can not pass through the bacterial cell wall. In order to use these starches as a carbon source, bacteria must secrete α-amylase and oligo-1,6-glucosidase into the extracellular space. These enzymes break the starch molecules into smaller glucose subunits which can then enter directly into the glycolytic pathway.

Procedure

On Starch agar (a simple nutritive medium with starch added). Inoculate of organism to be tested on to labeled plate and incubate it for 48 hours at 37°C. Following incubation, flood the surface of the plates with iodine solution with a dropper for 30 seconds. Pour off the excess iodine.

Examine for the clear zone around the line of bacterial growth.





Result Interpretation of Test and Examples

Positive test: A clear zone around the line of growth after addition of iodine solution indicates that the organism has hydrolyzed starch.

Negative test: A blue, purple, or black coloration of the medium

Bacillus subtilis is positive for starch hydrolysis (pictured below on the left).

The organism shown on the right is negative for starch hydrolysis (***E. coli***).

Bile Esculin Test

Principle :

Bile-esculin test is based on the ability of the group D streptococci and *Enterococcus* species, to hydrolyze esculin in the presence of bile.

Bile esculin agar contains **bile salts** to inhibit the growth of gram positive organisms other than enterococci and group D streptococci.
It also contains nutrients, **esculin,** and **ferric citrate**.

When an organism hydrolyzes the **glycoside esculin** to form **esculetin** and **dextrose**, the esculetin reacts with the ferric citrate to produce a dark brown or black phenolic iron complex.

Results

	
Enterococcus faecalis hydrolyzes esculin in the presence of bile and turns more than half the medium dark brown. This is a positive result.	*Streptococcus pyogenes* does not hydrolyze esculin in the presence of bile. No dark brown complex is formed. This is a negative result.

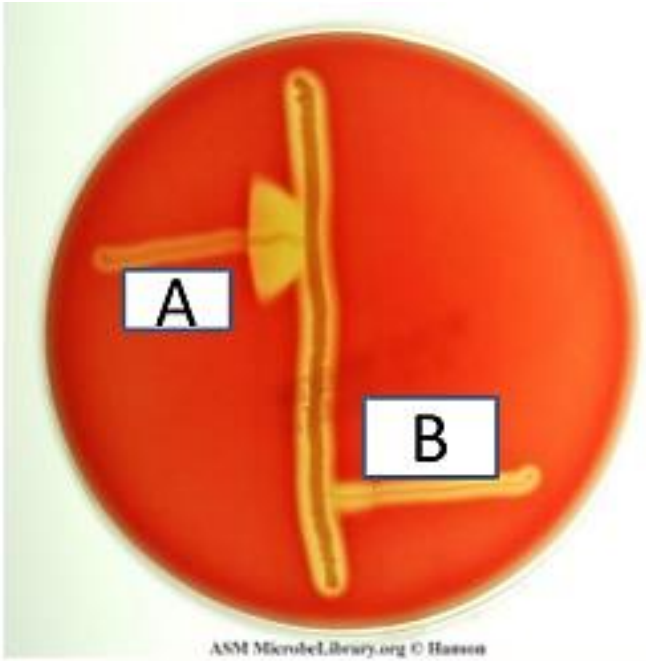
CAMP Test

CAMP factor is a diffusible, heat-stable protein produced by group B streptococci

This is a synergistic test between ***Staphylococcus aureus*** and ***Streptococcus agalactiae***. *S. agalactiae* produces CAMP factor

The two bacteria are streaked at 90^{o} angles of one another.

As a result, **an arrow** of beta-hemolysis is produced between the two streaks



Name of the test: CAMP test
Example A: Positive - Strept agalactiae (Arrow shaped)
Example B: Negative - Strept pyogens
Principle : Strept agalactiae produce CAMP factor (a diffusible extracelluar protein) that synergistically acts with the beta-lysin of *Staphylococcus aureus* and enhances the lysis of red blood cells.

Gelatinase Test

Principle of Gelatin Hydrolysis Test

This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinases that hydrolyze gelatin. The reaction occurs in two sequential steps: in first reaction gelatinases hydrolyze gelatin into polypeptides and then polypeptides are further converted into amino acids

The amino acids are taken up by the cell and used for metabolic purposes.

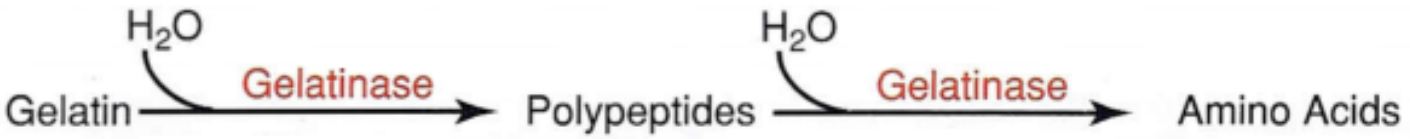


Fig. Gelatin Hydrolysis.Hydorlysis of gelatin by the gelatinase group of enzymes.

It distinguishes the gelatinase-positive, pathogenic ***Staphylococcus aureus*** from the gelatinase-negative, non-pathogenic ***S. epidermidis***

The test can also be used to differentiate genera of gelatinase-producing bacteria such ***Serratia*** and ***Proteus*** from other members of the family Enterobacteriaceae.

Procedure of Gelatin Hydrolysis Test

There are several methods for determining gelatin hydrolysis test. The most commonly used method is the nutrient gelatin stab method

-Stab a nutrient gelatin tube with bacteria and incubate for 24h at 37℃

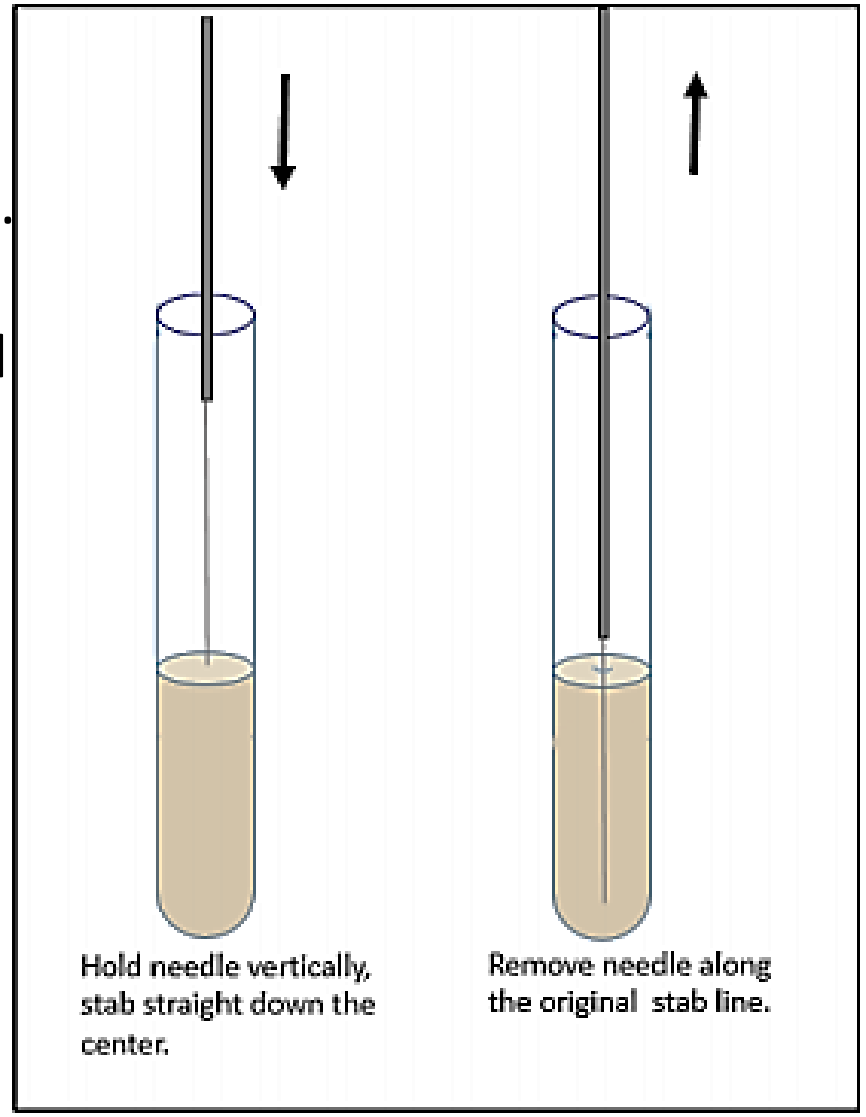
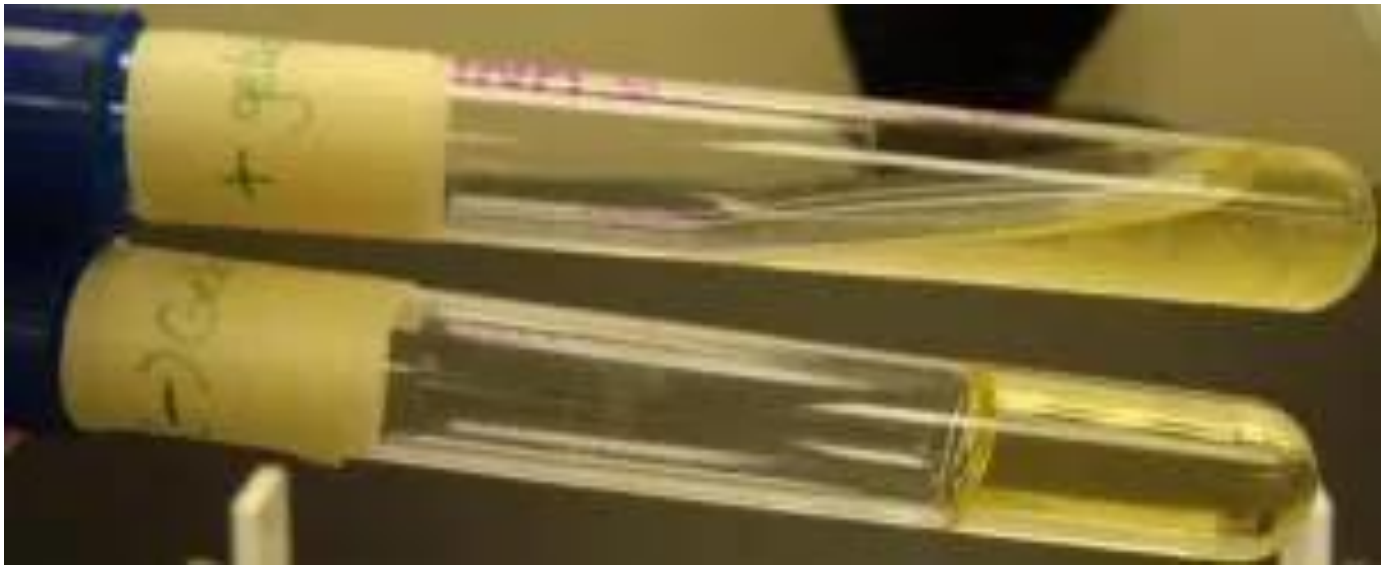


Figure Stab inoculation

Expected results

Positive: liquefaction of the inoculated tube even after exposure to cold temperature of ice bath or refrigerator (4°C)

Negative: Complete solidification of the inoculated tube



Thanks