Department of biology

((Microbiology))

stage 2

Fifth lecture

Laboratory diagnosis of bacteria

By

Msc. Zahraa Jawad Kadhim

Method of bacterial identification

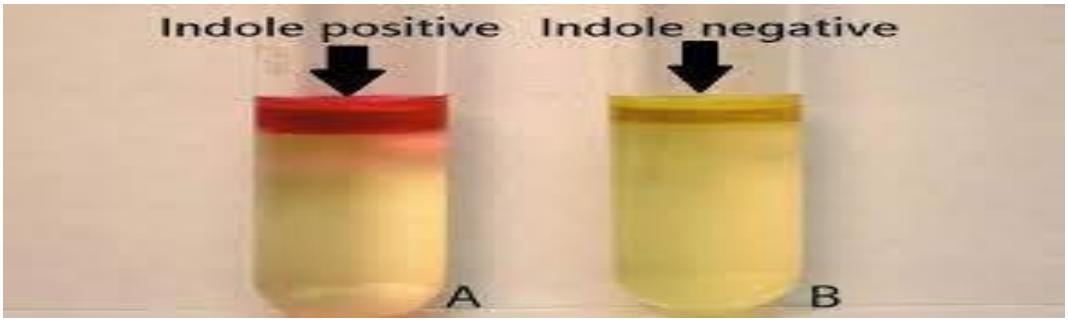
3. Biochemical reactions.

1-Sougar fermentation: a given carbohydrate may be fermented to a number of different end products depending upon the microorganism involved. These end products (alcohols, acids, gases, or other organic molecules) are characteristic of the particular microorganisms. For example, if fermenting bacteria are grown in a liquid culture medium containing the carbohydrate glucose, they may produce organic acids as by-products of the fermentation. These acids are released into the medium and lower its pH. If a pH indicator such as phenol red or bromcresol purple is included in the medium, the acid production will change the medium from its original color to yellow. Gases produced during the fermentation process can be detected by using a small, inverted tube, called a Durham tube within the liquid culture medium. **After adding the proper amount of broth, Durham tubes are inserted into each culture tube. During autoclaving, the air is expelled from the Durham tubes, and they become filled with the medium. If gas is produced, the liquid medium inside the Durham tube will be displaced, entrapping the gas in the form of a bubble**

Positive: The development of a yellow color in the medium is indicative of a positive carbohydrate fermentation reaction.

Negative: Lack of yellow color development is indicative of a negative carbohydrate fermentation reaction.

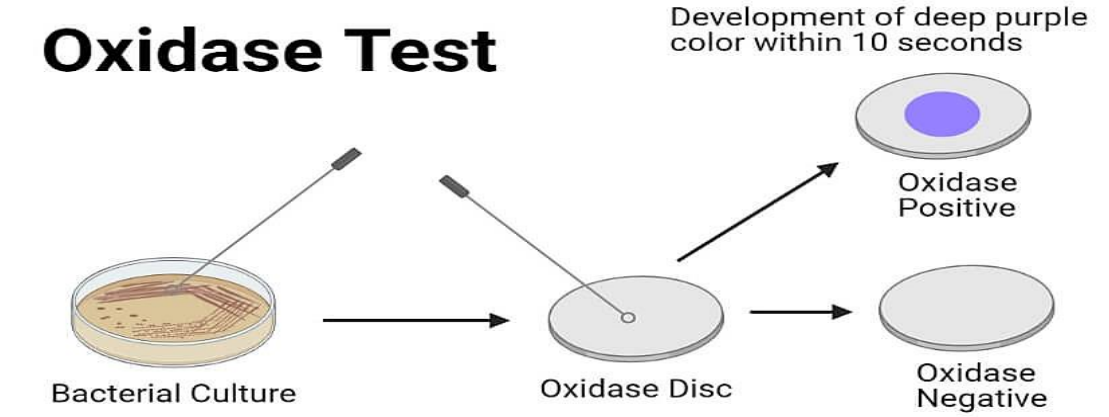
2-Indol test: The amino acid tryptophan is found in nearly all proteins. Bacteria that contain the enzyme tryptophanase can hydrolyze tryptophan to its metabolic products, mainly, indole, pyruvic acid, and ammonia. The bacteria use the pyruvic acid and ammonia to satisfy nutritional needs; indole is not used and accumulates in the medium. The presence of indole can be detected by the addition of Kovacs' reagent. Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium.



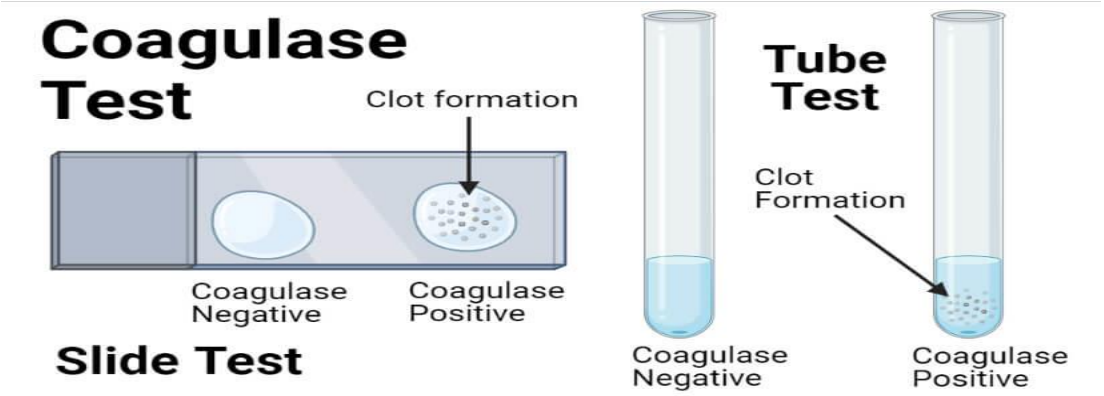
3- Catalase test:

The catalase enzyme serves to neutralize the bactericidal effects of hydrogen peroxide. Catalase expedites the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen ($2H_2O_2 + \text{Catalase} \rightarrow 2H_2O + O_2$). This reaction is evident by the rapid formation of bubbles. The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive Micrococcaceae from catalase-negative Streptococcaceae. The catalase test is also valuable in differentiating aerobic and obligate anaerobic bacteria, as anaerobes are generally known to lack the enzyme. In this context, the catalase test is valuable in differentiating aerotolerant strains of Clostridium, which are catalase negative, from Bacillus, which are catalase positive.

4-Oxidase test: The oxidase test identifies organisms that produce the enzyme cytochrome oxidase. Cytochrome oxidase participates in the electron transport chain by transferring electrons from a donor molecule to oxygen. The oxidase reagent contains a chromogenic reducing agent, which is a compound that changes color when it becomes oxidized.



5-Coagulase test: The coagulase test has traditionally been used to differentiate Staphylococcus auerus from coagulase-negative staphylococci. S. aureus produces two forms of coagulase (i.e., bound coagulase and free coagulase). Bound coagulase, otherwise known as "clumping factor", can be detected by carrying out a slide coagulase test, and free coagulase can be detected using a tube coagulase test.



6- Urease test :

Aim: To determine the ability of microbes to degrade urea by urease.

Principle:

•Urea is diamide carbonic acid often referred as carbamide.

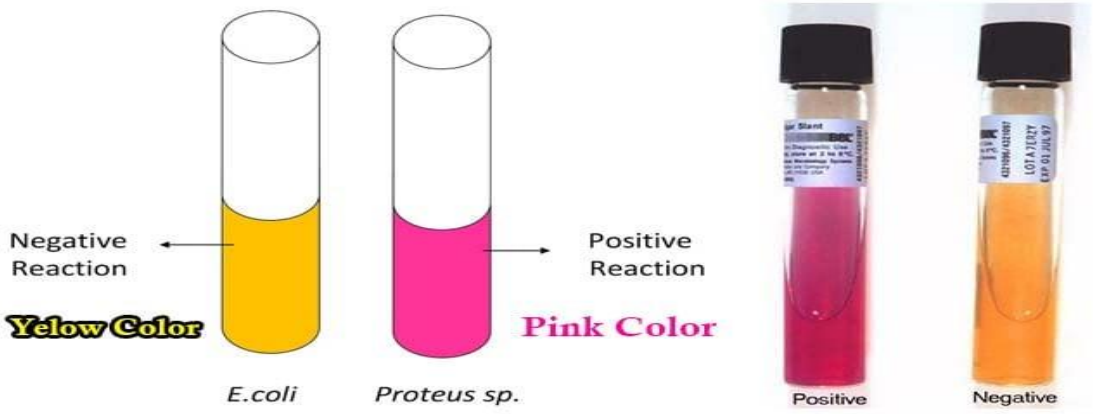
• The hydrolysis of urea is catalyzed by specific enzyme urease to yield 2 moles of ammonia. •Urease attacks the nitrogen and carbon bond in urea and forms ammonia.

• The presence of urease is detected, when the organisms are grown in urea broth.

• Medium containing the pH indicator phenol red.

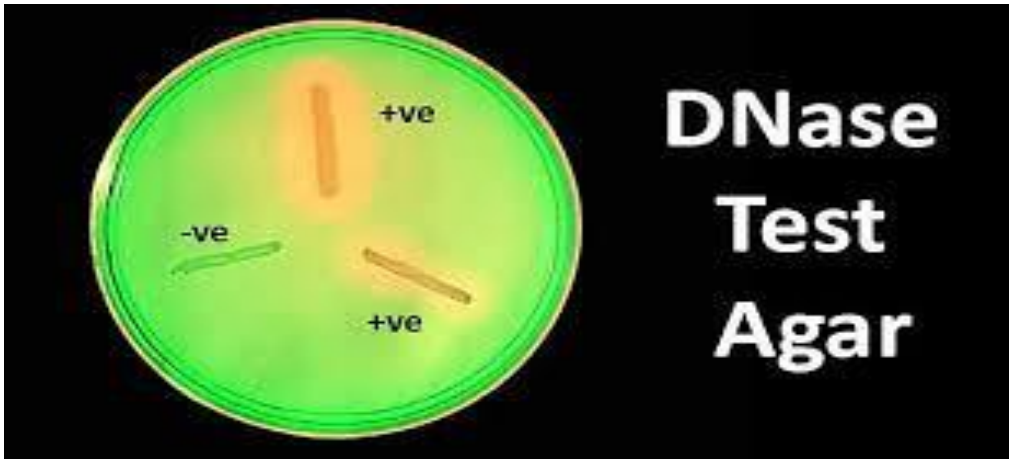
• Splitting of urea creates the alkaline condition which turns phenol red to deep pink in color.

• Mainly used for identification of Proteus spp. from other genus of lactose nonfermenting enteric organisms



7- DNAse test:

DNase agar is a differential medium that tests the ability of organism to produce an exoenzyme, called deoxyribonuclease or DNase, that hydrolyzes DNA. DNase agar contains nutrients for the bacteria, DNA, and methyl green as an indicator. Methyl green is a cation which binds to the negatively-charged DNA Deoxyribonuclease allows the organisms that produce it to break down DNA into smaller fragments. When the DNA is broken down, it no longer binds to the methyl green, and a clear halo will appear around the areas where the DNase producing organism has grown.



8-Gelatinase test :

liquefaction of gelatin is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin. Hydrolysis of gelatin indicates the presence of gelatinases. It distinguishes the gelatinase-positive, pathogenic Staphylococcus aureus from the gelatinase- negative, non-pathogenic S. epidermidis.