LAB 4:

GENUS: STAPHYLOCOCCUS

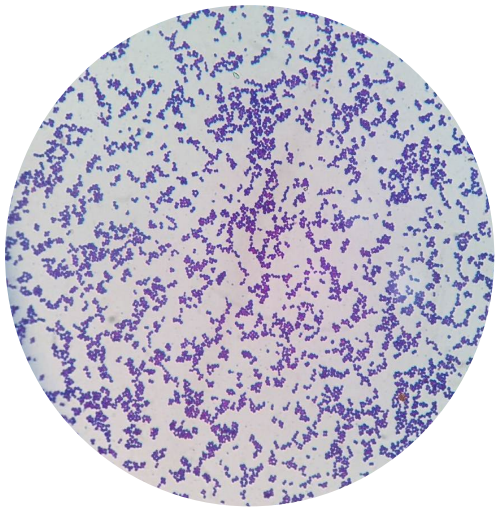
Classification

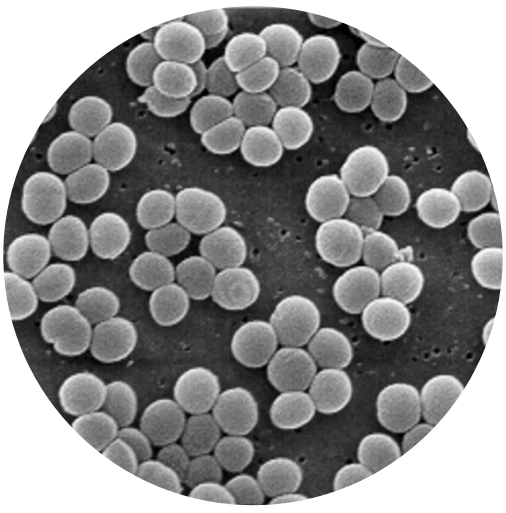
Domain: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcaceae
Genus: *Staphylococcus*

Taxonomy

The taxonomy is based on 16s rRNA sequences. There are about 40 species belonged to the genus Staphylococcus and that include11 groups
the common groups :
S. aureus group: – *S. argenteus, S. aureus, S. schweitzeri, S. simiae*
S. epidermidis group: – *S. capitis, S. caprae, S. epidermidis, S. saccharolyticus*
S. haemolyticus group: – *S. devriesei, S. haemolyticus, S. hominis*
S. saprophyticus group: – *S. arlettae, S. cohnii, S. equorum, S. gallinarum, S. kloosii, S. leei, S. nepalensis, S. saprophyticus,*

General characteristics





Staphylococcus is a Gram-positive,0.8-1 µ in diameter. Under the microscope, they appear as spherical (cocci), and form a grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms (capable of growth both aerobically and anaerobically). non-capsulated, non –motile, non-spore former. Some members are normal flora of the skin and mucous membrane.

Species of Staphylococci are initially differentiated by the coagulase test and are classified into two groups: the coagulase-positive and coagulase-negative staphylococci (CNS).

Staphylococcus aureus

It is human major pathogen causes infection ranging from minor skin infections to sever infections (septicemia).

Infections caused by Staph. aureus:

1- skin infection including: acne, impetigo, infection of surgical wounds.
2- Bacteremia: endocarditis, meningitis, osteomyelitis.
3- Pneumonia.
4- Disease associated with toxin production:
*Food poisoning (Enterotoxin).
* Staphylococcal scalded skin syndrome (Exfoliative toxin).
* Toxic Shock Syndrome Toxin-1 (TSST-1).

Cultural and Characteristics and Biochemical Reactions:

Optimum temperature for growth is 37°C (range being 12-44°C). Optimum pH is 7.5. They can grow readily on ordinary media. colonies are 1-3 mm in diameter and have a smooth glistening surface, an entire edge, a soft butyrous consistency and an opaque, pigmented appearance. Most strains produce golden-yellow pigment.

- Blood agar: Colonies are surrounded by a β-hemolysis zone.
- Mannitol salt agar: Yellow-colored colonies (because of Mannitol fermentation.

- Catalase:positive (meaning it can produce the enzyme catalase),
- Oxidase: negative.

- Coagulase: positive.

- DNAse: positive.

Staphylococcus epidermidis:

Staphylococcus epidermidis It is part of the human normal flora, typically the skin and less commonly in mucosa. It is a facultative anaerobic bacterium. Although S. epidermidis is not usually pathogenic, but patients with compromised immune systems are at risk of developing infection. These infections are generally hospital-acquired.

S. epidermidis is a concern for people with catheters or other surgical implants because it is known to form biofilms that grow on these devices.

S. epidermidis forms white, raised, cohesive colonies about 1–2 mm in diameter after overnight incubation, and is not hemolytic on blood agar. It is a catalase-positive, coagulase-negative, facultative anaerobe that can grow by aerobic respiration or by fermentation. Some strains may do not have ability for fermentation.

Biochemical tests indicate this microorganism also carries out a weakly positive reaction to the nitrate reductase test. It is positive for urease production, oxidase negative, and can use glucose, sucrose, and lactose to form acid products. In the presence of lactose, it will also produce gas. *S. epidermidis* does not possess the gelatinase enzyme, so it cannot hydrolyze gelatin. It is sensitive to novobiocin.

Staphylococcus saprophyticus

S. saprophyticus: belonging to the coagulase-negative genus *Staphylococcus.* Concorded as common cause of community-acquired urinary tract infections especially in young women. *S. saprophyticus* is differentiated from *S. epidermidis* by testing of susceptibility to the antibiotic novobiocin. *S. saprophyticus* is

novobiocin-resistant, while *S. epidermidis* is novobiocin-sensitive. *S. saprophyticus* are DNase negative.

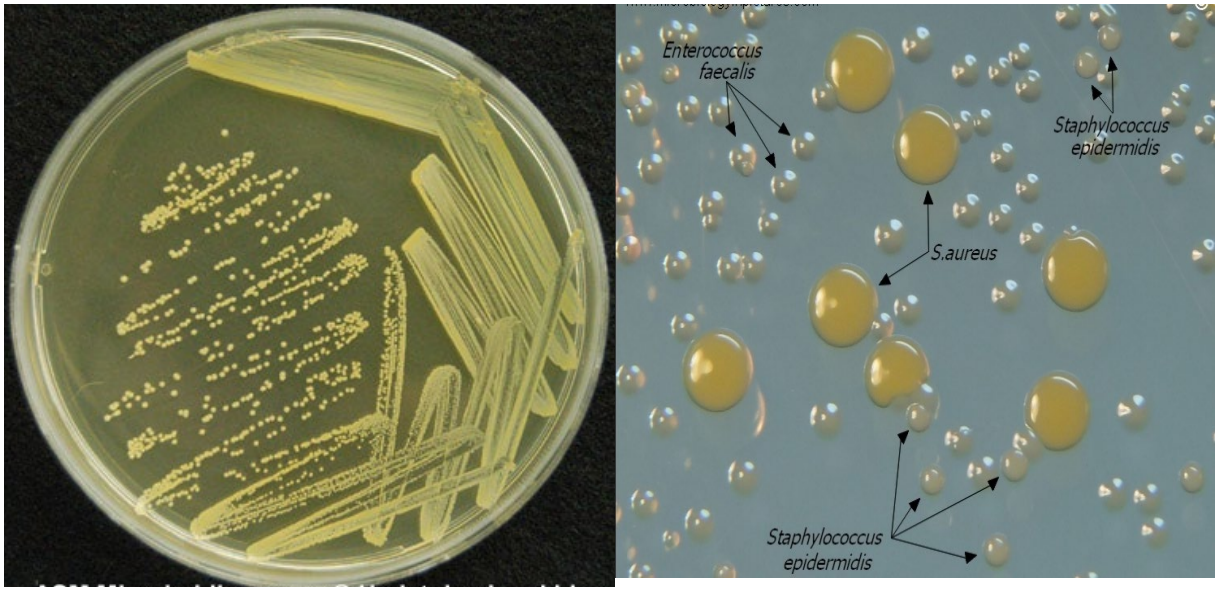
Specimens

Blood, urine, pus, wound swab, burn swab, and tracheal aspirate.

Lab. Diagnostic test

1- Gram stain: gram positive, cocci, grape -like clusters

2- Nutrient agar: colonies morphology: circular, entire, convex, golden yellow or white.



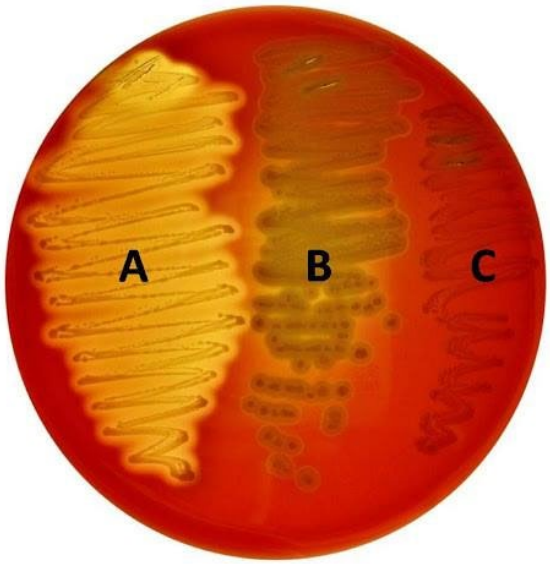
3- Blood agar: for hemolysis: circular, entire, convex, yellow or white or golden.

Blood Agar Plates (BAP) This is a differential medium contains 5% sheep red blood cells. Useful to detect the ability of an organism to produce hemolysin, enzymes that damage/lyse red blood cells (erythrocytes). The degree of hemolysis by hemolysin is helpful in differentiating members of the genera *Staphylococcus*, *Streptococcus* and *Enterococcus*.

* Beta-hemolysis (β): complete hemolysis. It is characterized by a clear (transparent) zone surrounding the colonies. *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus agalactiae* are beta-hemolytic

* Alpha-hemolysis (α): Partial hemolysis. Colonies typically are surrounded by a green, opaque zone. *Streptococcus pneumoniae* and *Streptococcus mitis* are alpha-hemolytic

* Gamma-hemolysis (ɣ): no hemolysis occurs. There are no notable zones around the colonies. *Staphylococcus epidermidis* is gamma-hemolytic.

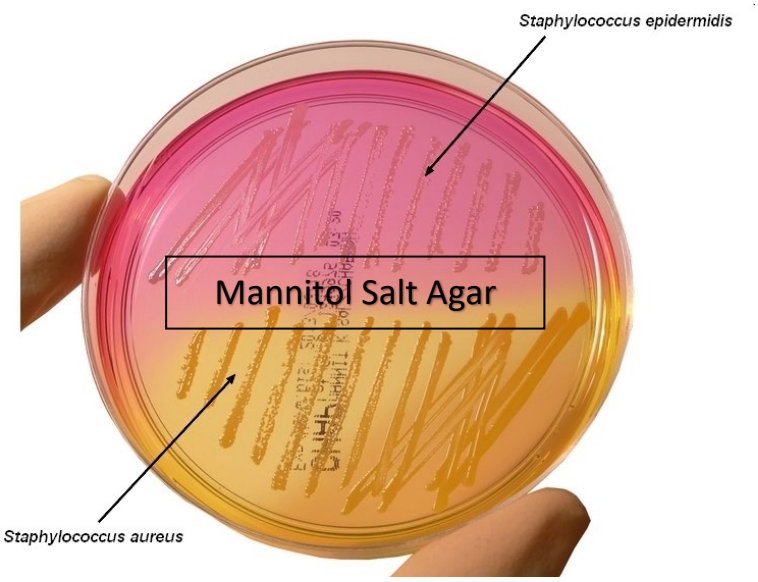


A= (β) Beta Hemolysis

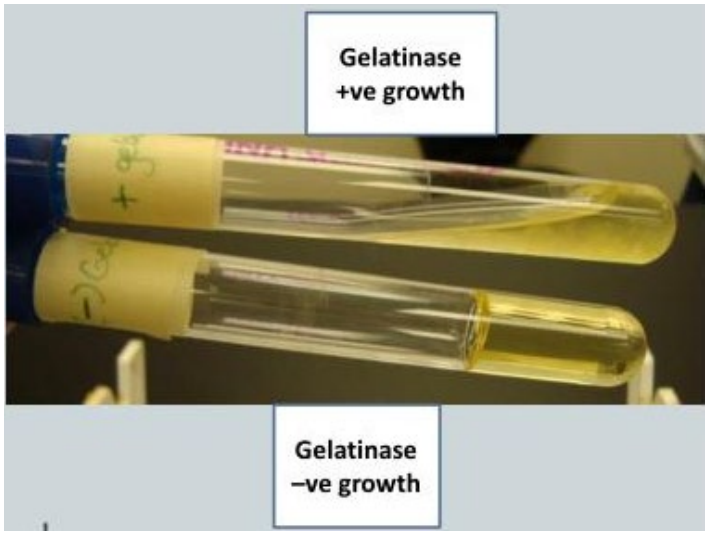
B= (α) Alpha Hemolysis

C= (ɣ) Gamma Hemolysis

4- Mannitol Salt Agar (MSA): is a selective and differential medium. The high concentration of salt (7.5%) selects for members of the genus *Staphylococcus*, since they can tolerate high saline levels. Organisms from other genera may grow, but they typically grow very weakly. MSA also contains the sugar mannitol and the pH indicator phenol red. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow. Most pathogenic *staphylococci*, such as *Staphylococcus aureus*, will ferment mannitol. Most non-pathogenic *staphylococci* will not ferment mannitol. *S. aureus* appear yellow while *S. epidermidis* appear red.



5- Gelatin hydrolysis 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*.

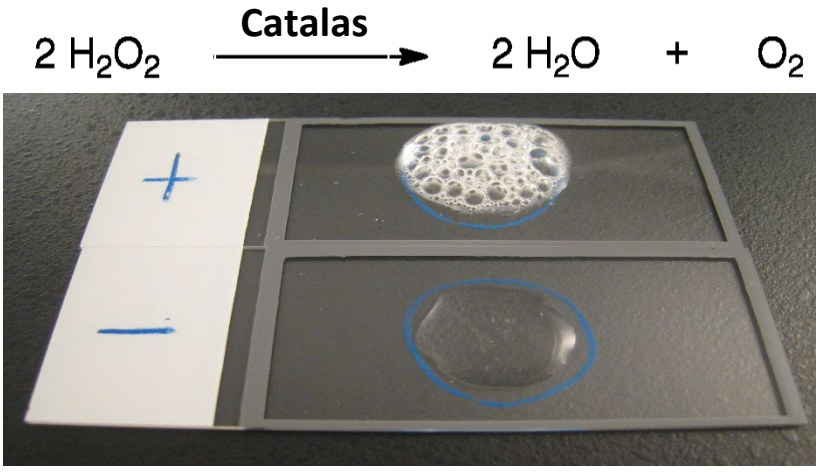


6- Catalase test: This test is used to identify organisms that produce the catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The sample on the right below is catalase positive.

The *Staphylococcus* spp. and the *Micrococcus* spp. are catalase positive. The *Streptococcus* and *Enterococcus spp. are* catalase negative.

Procedure

a. Place a small amount of growth from your culture onto a clean microscope slide. If using
colonies from a blood agar plate, be very careful not to scrape up any of the blood agar—
blood cells are catalase positive and any contaminating agar could give a false positive.
b. Add a few drops of H2O2 onto the smear. If needed, mix with a toothpick. DO NOT use
a metal loop or needle with H2O2; it will give a false positive and degrade the metal.
c. A positive result is the rapid evolution of O2 as evidenced by bubbling.
d. A negative result is no bubbles or only a few scattered bubbles.
e. Dispose of your slide in the biohazard glass disposal container. Dispose of any toothpicks in the Pipet Keeper.



NOTE: catalase test not done on blood agar.

7- 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

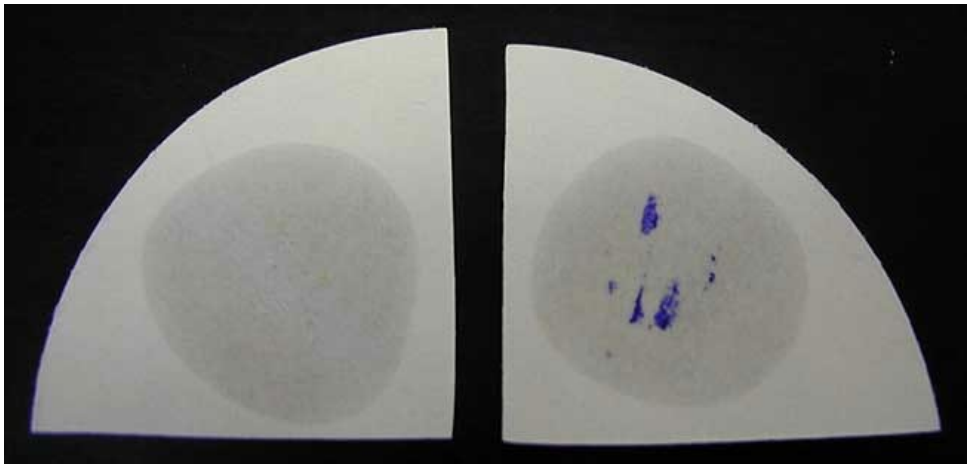
chromogenic reducing agent, which is a compound that changes color when it becomes oxidized.

Procedure:

a- A strip of filter paper is soaked with a little freshly made 1% solution of the reagent (1% tetramethyl-p-phenylenediamine dihydrochloride)

b- A spot of culture is rubbed on it with a platinum loop

c- A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a “delayed positive” reaction by coloration in 10-60 seconds, and a negative reaction by absence of coloration or by coloration later than 60 seconds.



- ve Oxidase Test + ev

8- 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).

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

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 drop to make two thick suspensions.
3- Add a drop of human or rabbit plasma to one of the suspensions, and mix gently.
4- Look for clumping of the organisms within 10 seconds.
No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

NOTE:

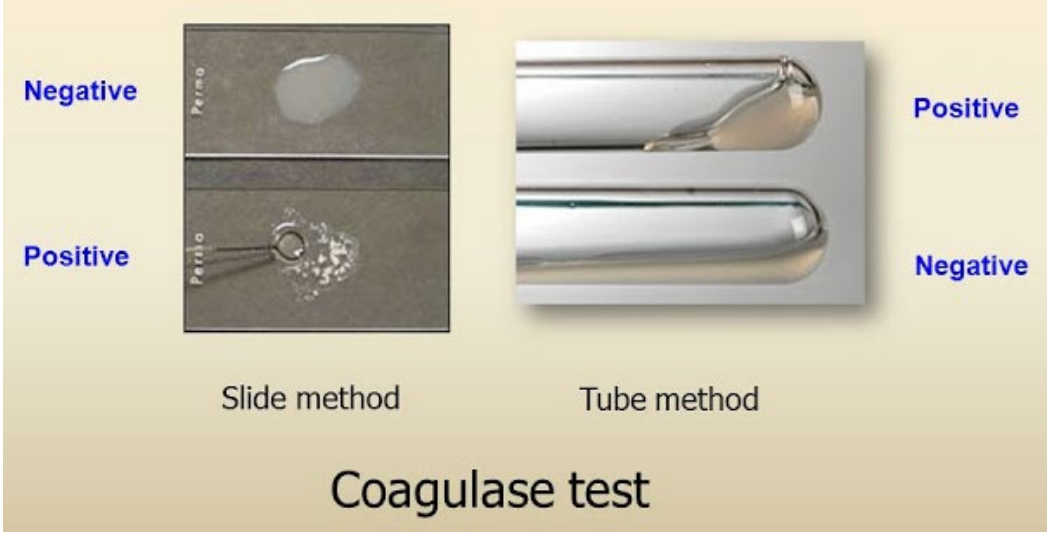
*The slide test should be read very quickly, as false positives can occur.
*The slide test should not perform with organisms taken from high-salt media such as Mannitol Salt Agar, as the salt content can create false positives.

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.

Tube Test (to detect free coagulase)

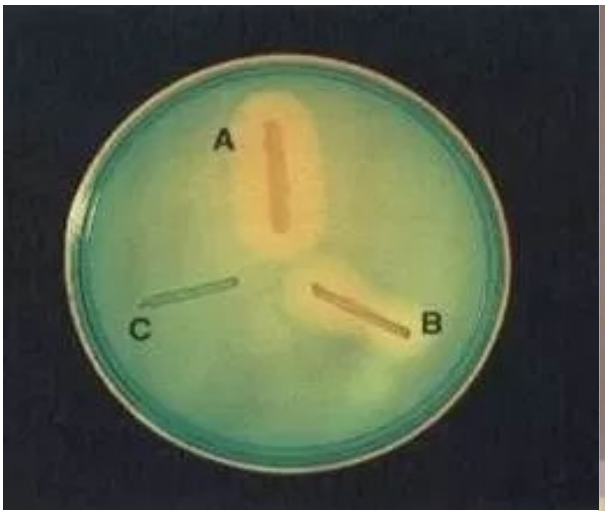
1-Dilute the plasma 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline).
2- Take 3 small test tubes and label as T (Test), P (Positive Control) and N (Negative Control). Test is a (18-24) hour broth culture, Positive control is 18-24 hr. *S. aureus* broth culture and Negative 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 Degree Celsius.

6- Examine for clotting after 1 hours. If no clotting has occurred, examine at 30 minutes intervals for up to 6 hours.

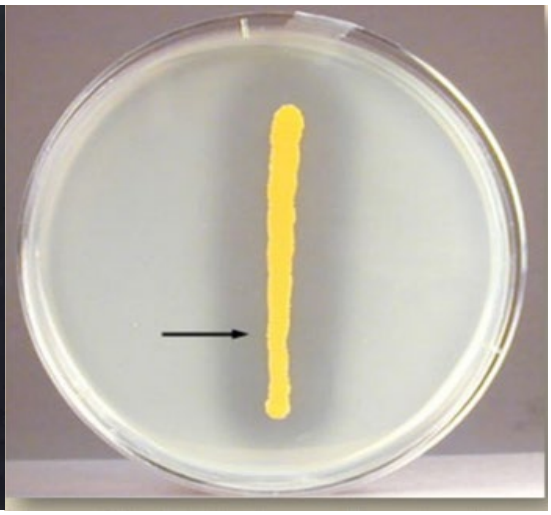


9- DNase test: DNase agar is a differential medium that tests the ability of an organism to produce an exoenzyme, called deoxyribonuclease or DNase, that hydrolyzes DNA. DNase agar contains nutrients for the bacteria, DNA, and methyl green as an indicator. Methyl green is a cation which binds to the 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.

If we using DNase agar without indicator, Flood the plate with 1N Hydrochloric Acid. Leave the plate to stand for a few minutes to allow the reagent to absorb into the plate. Decant excess hydrochloric acid and then examine the plate within 5 minutes against a dark background.



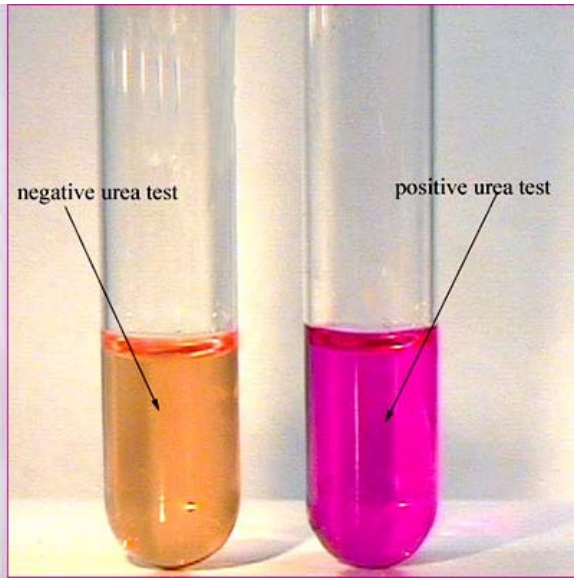
DNase agar with indicter



DNase agar without indicator

10-Urease test: This test is used to identify the bacteria capability to hydrolyzing urea by urease. The hydrolysis of urea forms a weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink.

The test can preform in broth or slant urease medium by inoculating the tested organism in the by aseptic way.



Test	*S. aureus*	*S. epidermidis*	S. *saprophyticus*
Pigment	Golden, yellow Cream	White	White
Mannitol growth	Positive	Positive	Positive
Mannitol fermentation	Positive	Negative	Variable Pos/neg
Catalase	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative
Coagulase	Positive	Negative	Negative
DNase	Positive	Negative	Negative
Urease	Positive	Positive	Positive
Gelatin liquefaction	Positive	Negative	Positive
Hemolysis	Beta-hemolysis	Non-hemolytic	Non-hemolytic
Novobiocin susceptibility	S	S	R
O/F test	Oxidative &fermentative	Oxidative &fermentative	Oxidative &fermentative