Practical Pathogenic Bacteria

LAB: 2

Genus: *Staphylococcus*

General Characteristics

- It is a Gram-positive bacterium.

- This genus includes: *S. aureus*, *S. epidermidis*, *S. saprophyticus*

- Microscopic examination: Spherical (cocci), arranged in grape-like clusters.

-Facultative anaerobic (capable of growing aerobically and anaerobically).

- Non-motile, non-spore forming, some species have capsule.

- Catalase: positive, oxidase: negative, coagulase: variable, DNase: variable, mannitol fermentation: variable.

- Many species cannot cause disease and exist normally on the skin and mucous membranes of human and other animals. They also consist a small component of the soil microbiome.

- It can grow in the presence of 7.5% NaCl solution.

- Blood hemolysis: Beta hemolysis *S.aureus*

Laboratory Specimens

- Urine.

- Blood.

- Stool.

- Swabs from ear, nose and eye.

- Acne, burns and wounds.

- Seminal fluid.

Laboratory Diagnostics

1- Gram staining: purple grape-like clusters.

2- Catalase test.

3-Oxidase test.

4- Coagulase test.

5- Motility test.

6- DNase test.

7- Growth on milk agar for pigments.

(*S.aureus* appeared as glistening orange convex colonies due to staphyloxanthin production (a golden carotenoid pigment)

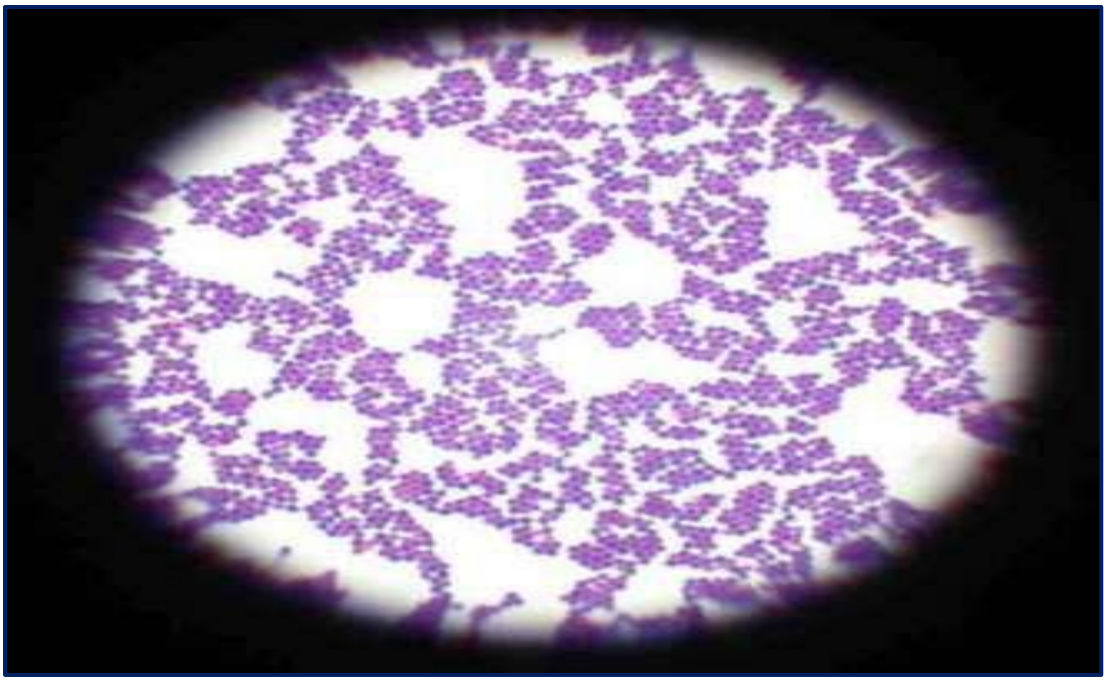
8- Growth on blood agar for blood hemolysis

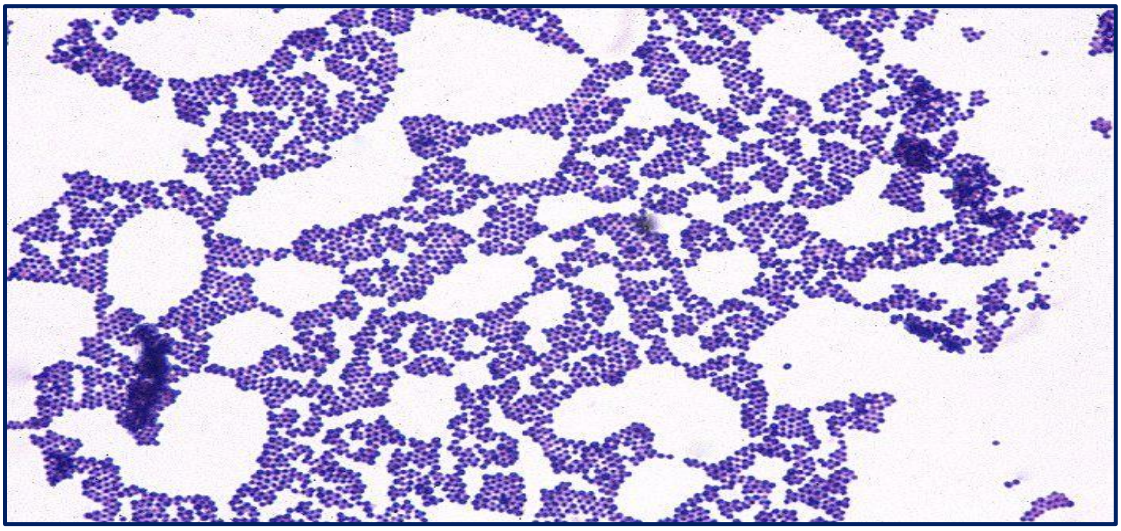
9- Growth on Mannitol salt agar for mannitol fermenting

Gram staining procedure

The Gram staining process includes four basic steps:

1. **Applying a primary stain (crystal violet) for 1 min.**
2. **Adding a mordant (Gram's iodine) for 1 min.**
3. **Rapid decolorization with ethanol for 10-30 secs.**
4. **Applying a counter staining (safranin) for 0.5-1 min.**





***Staphylococcus* spp = Cocci, Grape-like clusters and G+ve (purple)**

Mannitol salt agar

Chapman agar or mannitol salt agar is a selective medium used for the isolation, enumeration and differentiation of *Staphylococcus* species from clinical, food, antiseptic and cosmetic samples.

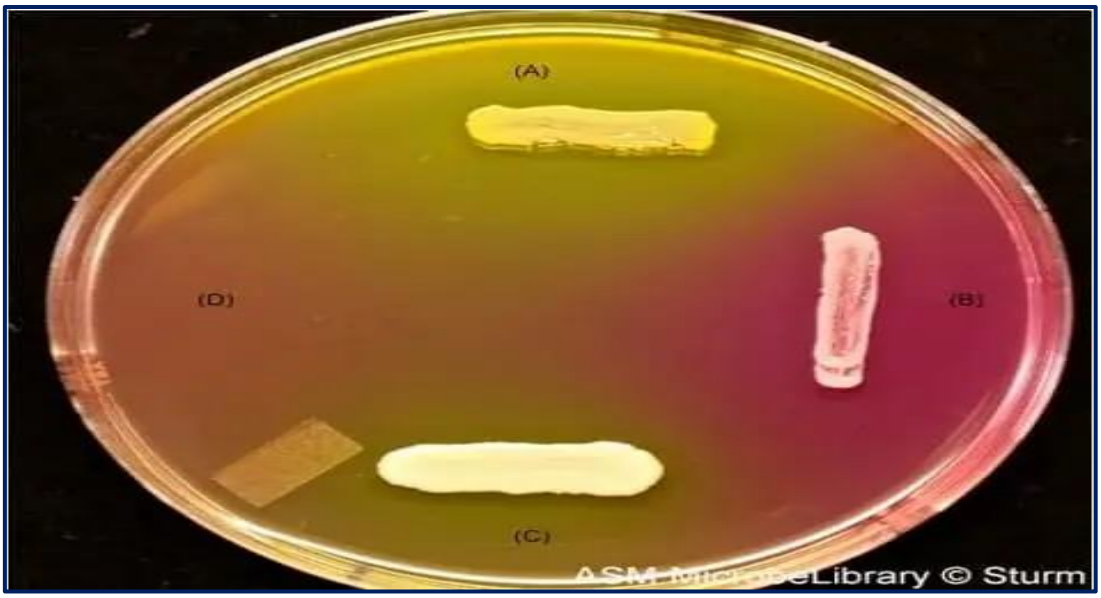
This medium is both selective and differential medium. It will select the bacteria that can live in high concentrations of salt (sodium chloride) and can ferment mannitol. Mannitol fermenter species appear as yellow colonies which is considered as a guide for the diagnosis.

Principle of Mannitol Salt Agar

-The selectivity of this medium is based on the presence of sodium chloride (7.5%) which inhibits most Gram-negative and Gram-positive bacteria.

-The differentiation of this medium is based on the ability of bacterial species to ferment or not the sugar mannitol. If the bacteria can ferment the sugar, this induces acidification which leads to decrease the pH levels below 6.9, and change of the pH indicator phenol red from red to yellow.

- Mannitol fermenter species is *Staphylococcus aureus* which form pigmented colonies surrounded by a yellow area while a non-fermenter species is *Staphylococcus epidermidis* results in a red to pink area due to the degradation of the peptone.

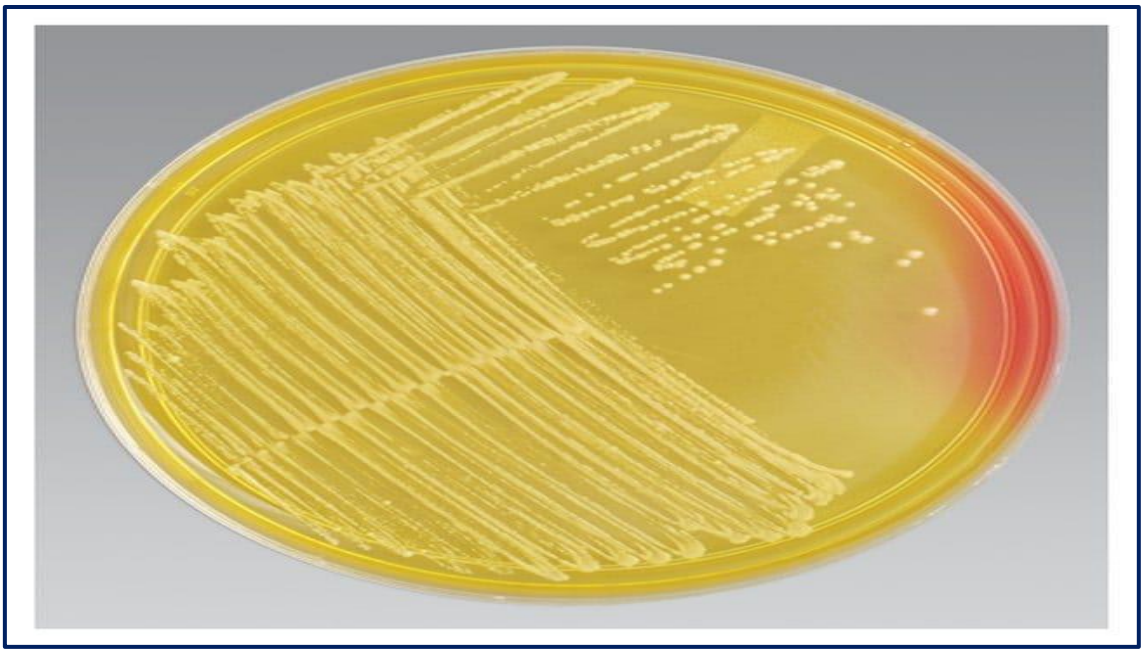


(A) *Staphylococcus aureus*: a large yellow halo around the growth indicates the fermentation of mannitol.

(B) *Staphylococcus epidermidis*: the bacterial growth but not the color change of the medium indicates no fermentation of mannitol.

(C) *Staphylococcus saprophyticus*: a small yellow halo around growth indicates fermentation of mannitol. (10% of *S. saprophyticus* can ferment mannitol)

(D) *E. coli*: no growth. Inhibited by the 7.5% Nacl



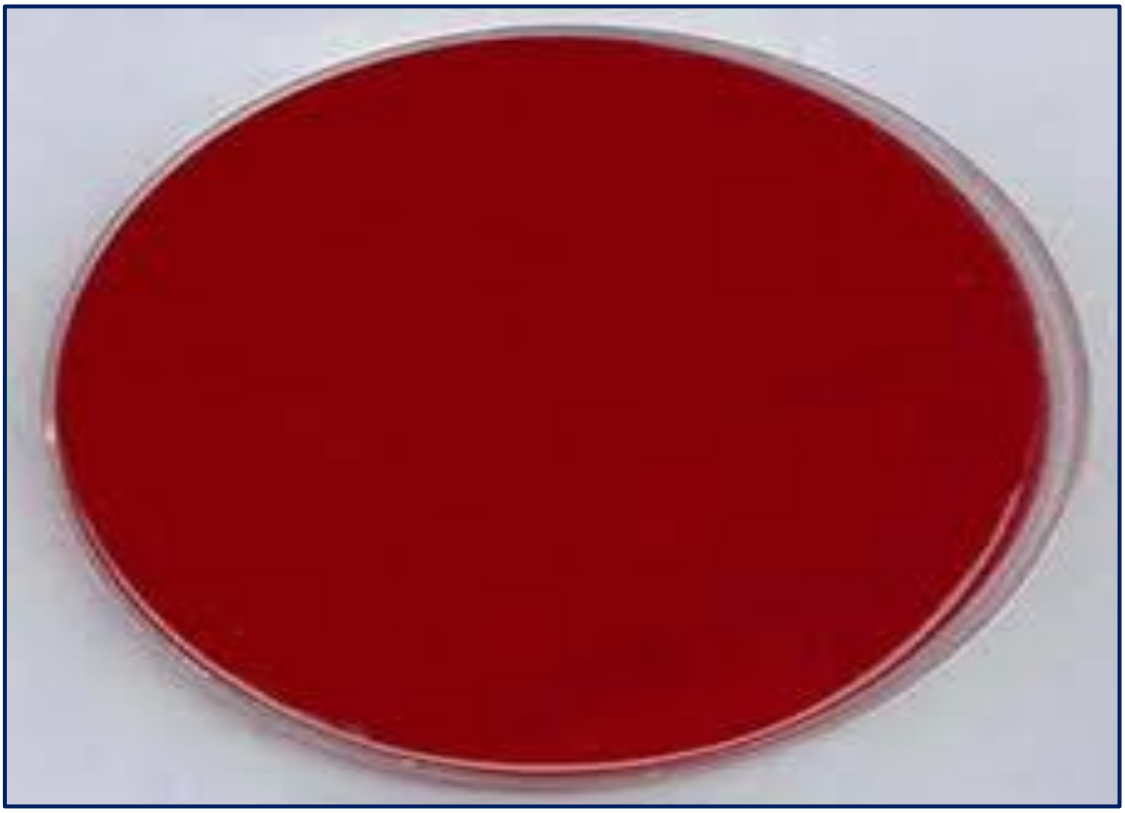
Staphylococcus aureus **on mannitol salt agar**

Staphylococcus epidermidis **on mannitol salt agar**

Blood Agar

- Blood agar is a selective enriched medium often used to grow fastidious bacteria.

- It is also a differential medium used to differentiate bacteria based on their hemolytic properties Beta, Alpha and Gamma hemolysis. In the U.S., blood agar is usually prepared from tryptic soy agar or Columbia agar base with 5% sheep blood.



DNase Test

- This test is presumptively used to differentiate *Staphylococcus aureus* which produces the enzyme deoxyribonuclease from other Staphylococci which do not produce deoxyribonuclease (DNase).

- *Staphylococcus aureus* possesses a heat-stable enzyme, a thermonuclease. To detect this enzyme, first the organisms are destroyed by heat and then the free DNase reacts with the medium.

- This test is also given positive by *Vibrio, Helicobacter, Moraxella, Serratia, and Aeromonas*.

Principle

- This test determines the ability of an organism that produce DNase.

- DNase are extracellular endonucleases that cleave DNA and release free nucleotides and phosphate.

- To detect these enzymes, DNase agar using no indicators or various indicators (toluidine blue or methyl green) are used to detect the hydrolysis of DNA.

- In DNase agar without indicator, the hydrolysis of DNA is observed by a clearing of the agar after addition of HCL (oligonucleotides dissolves in acid but DNA salts are insoluble). The acid precipitates unhydrolyzed DNA making the medium opaque. Therefore, DNase producing colonies hydrolyze DNA and produce a clear zone around the growth.

In case of DNase agar with methyl green, DNA combines with methyl green (act as cation) to produce mint green color. When the DNA is hydrolyzed, the complex is released and the free methyl green is colorless at pH 7.5. So, the clear halo is appeared around the areas where DNase producing organism grow.

When toluidine blue O (TBO) is added to the DNase agar, a complex is formed with the DNA, which changes structure when DNA is hydrolyzed, resulting in a bright pink color.

Uses

1. **Used to determine the ability of an organism to hydrolyze deoxyribonucleic acid.**
2. **Used to differentiate *Staphylococcus aureus* which produces the enzyme deoxyribonuclease from other Staphylococci which do not produce DNase.**
3. **Particularly useful if plasma is not available to perform coagulase test or when the result of coagulase tests is difficult to interpret.**

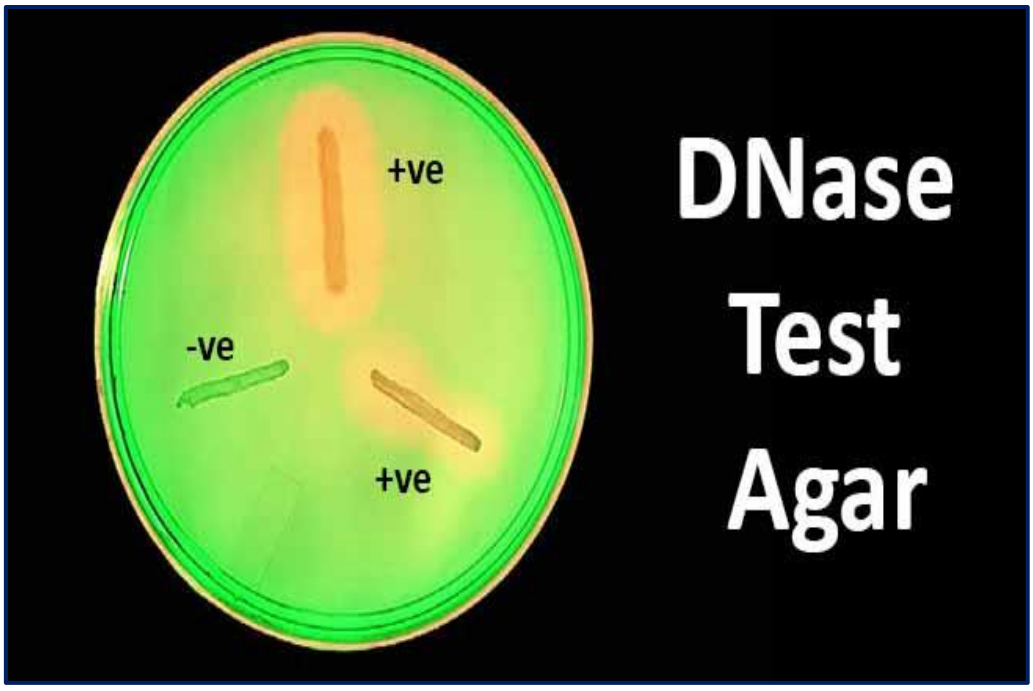
Procedure

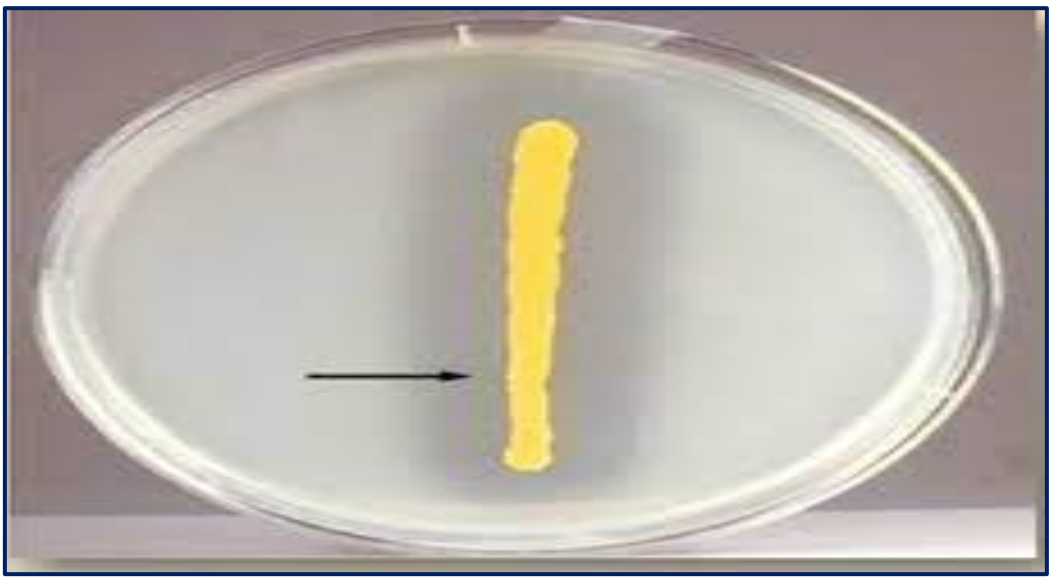
DNase test method

1. **Using a sterile loop, several colonies from an 18-24 hrs culture are picked.**
2. **Inoculate the test and control organism in each test area.**
3. **Incubate the plate at 35-37°C for 24 hours.**
4. **After incubation observe the color change in DNase with methyl green.**
5. **In DNase agar without indicators:**

- **Flood the surface of agar with 1N HCL solution. Tip off the excess acid.**

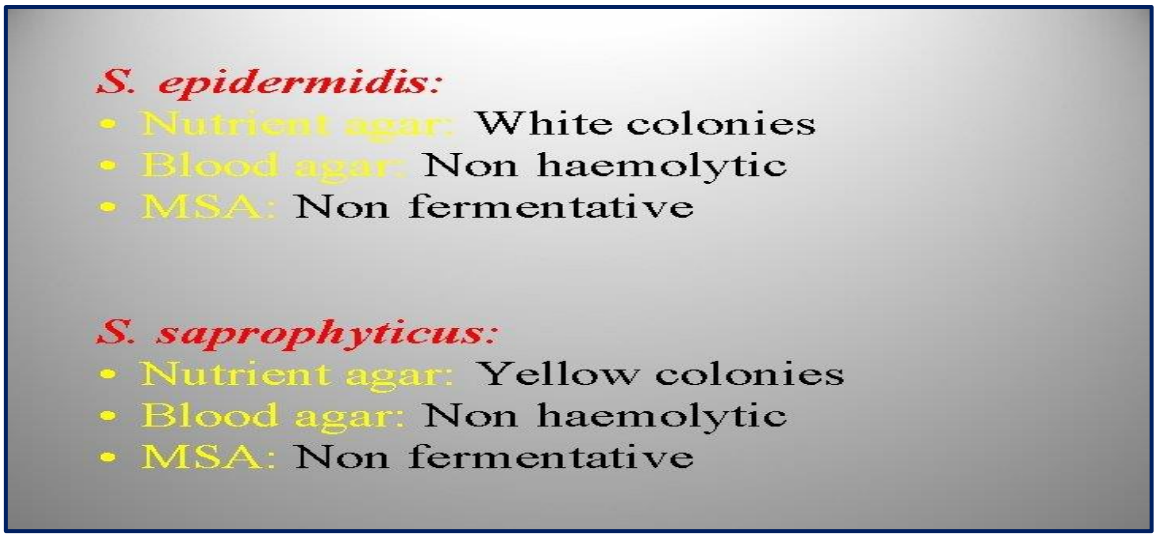
- **Allow the reagent to absorb into the plate.**
- **Observe for clear zone around the colonies within 5 mins.**





DNase test positive : *Staphylococcus aureus*

DNase test negative : *Staphylococcus epidermidis*



Differentiation between *Staphylococcus* species

Tests	*S.aureus*	*S.epidermidis*	*S.saprophyticus*
Gram stain	Gram+ve cocci	Gram+ve cocci	Gram+ve cocci
Catalase	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative
Blood agar	Beta	Gamma	Gamma
Coagulase	Positive	Negative	Negative
DNase	Positive	Negative	Negative
Mannitol fermentation	Ferment mannitol	Dose not ferment	Dose not ferment
Novobiocin sensitivity	S	S	R
Colony	Golden	White	Light yellow