



# 460 MBIO

# Medical Bacteriology

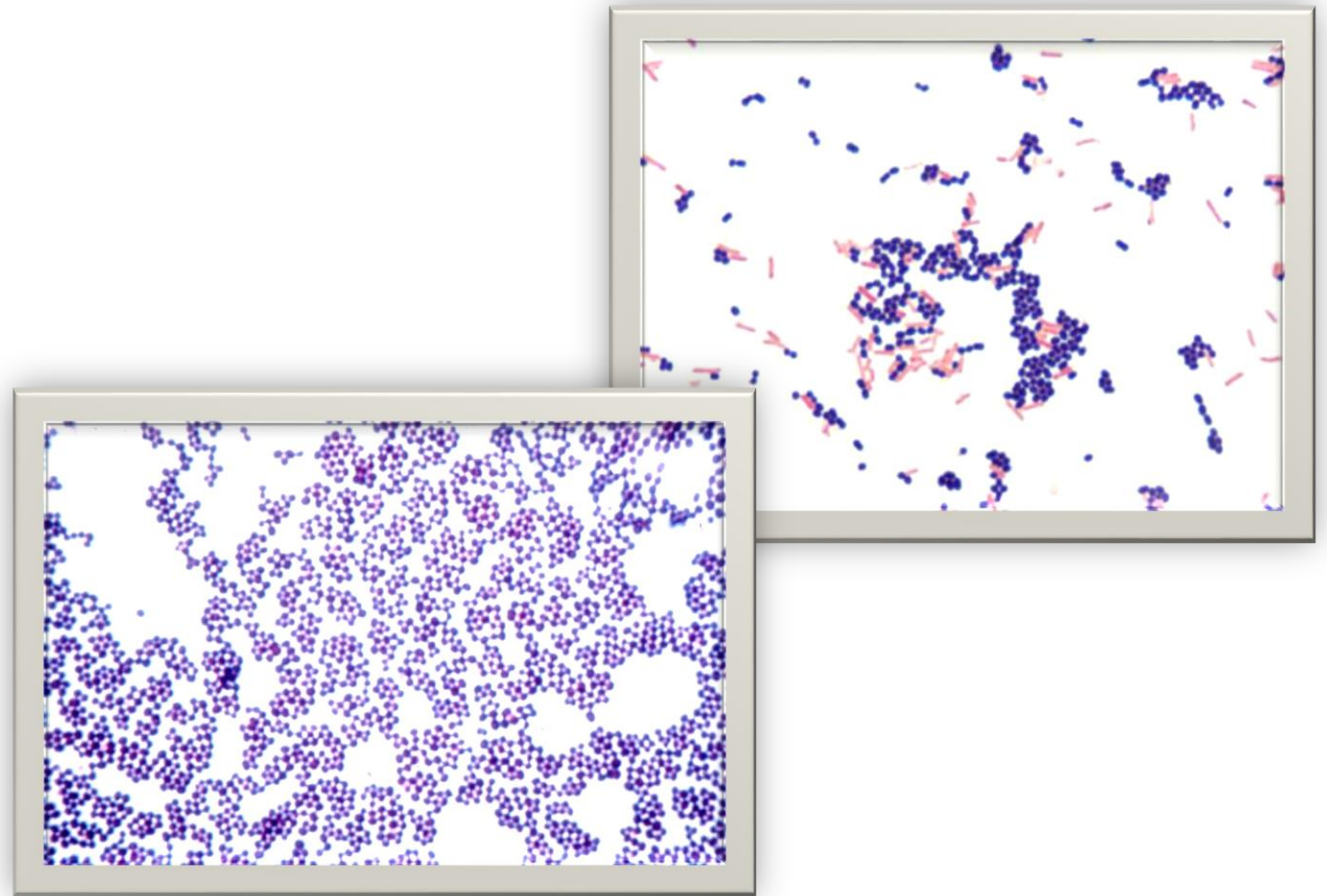
Aljawharah F. Alabbad

2017

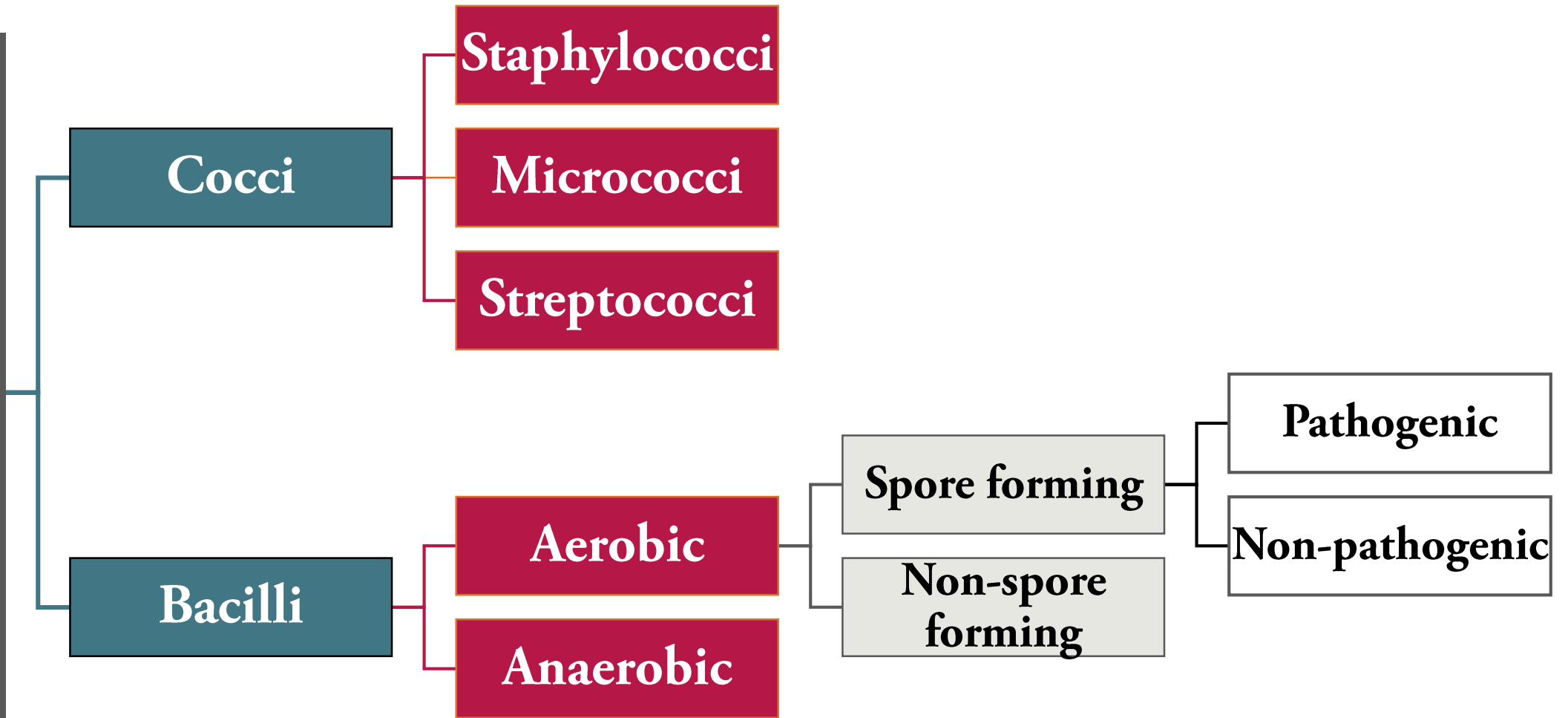


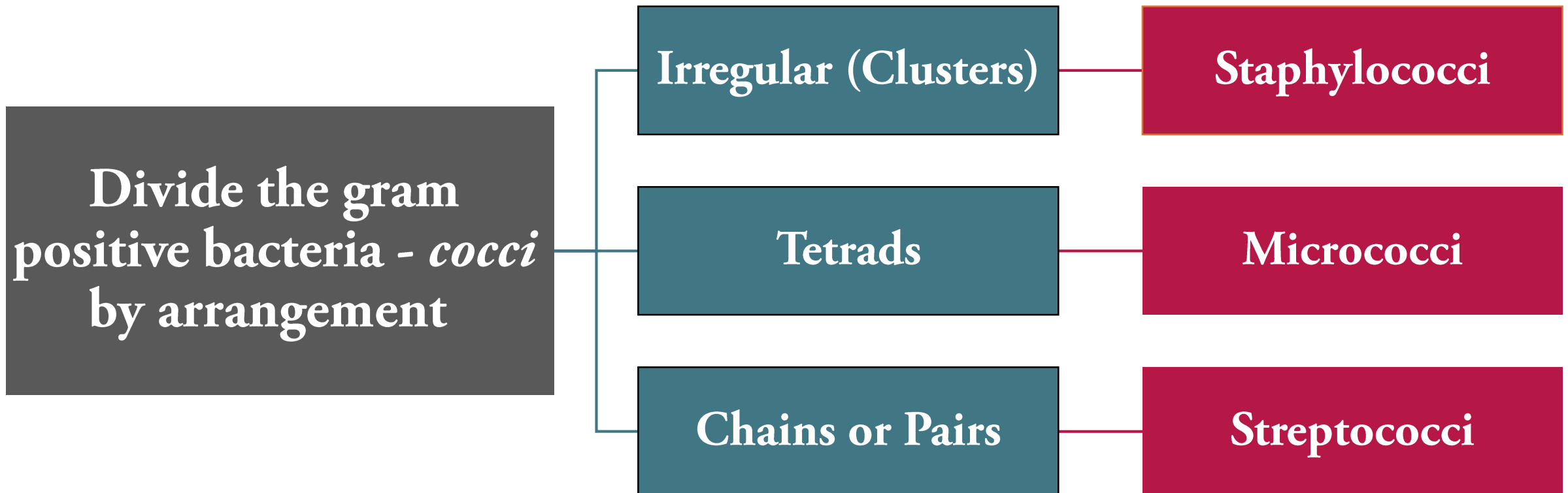
# ❖ Divided bacteria according to the following :

1. Reaction to gram.
2. Shape.
3. Arrangement.
4. Spore forming.
5. Oxygen needs.

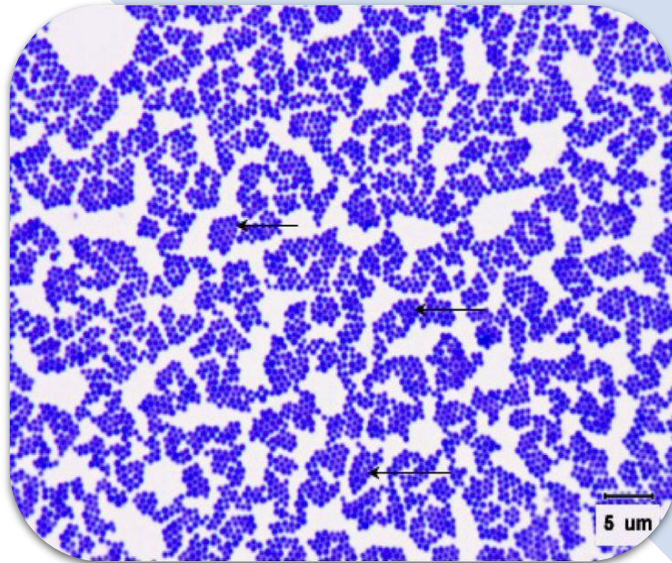
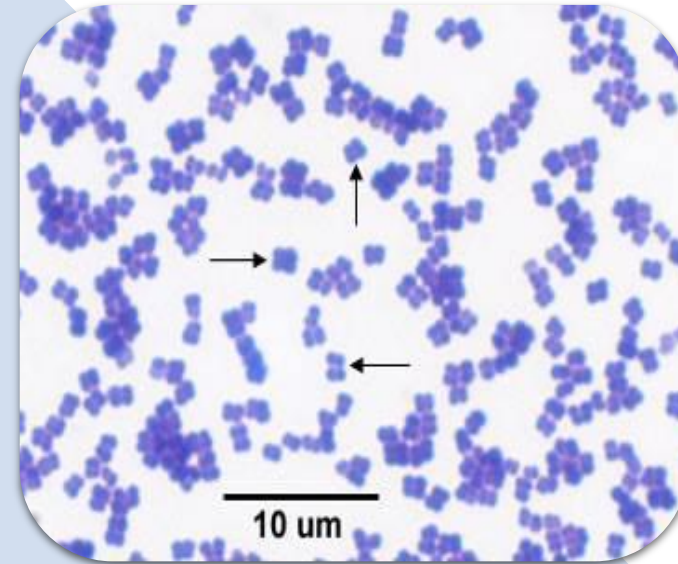
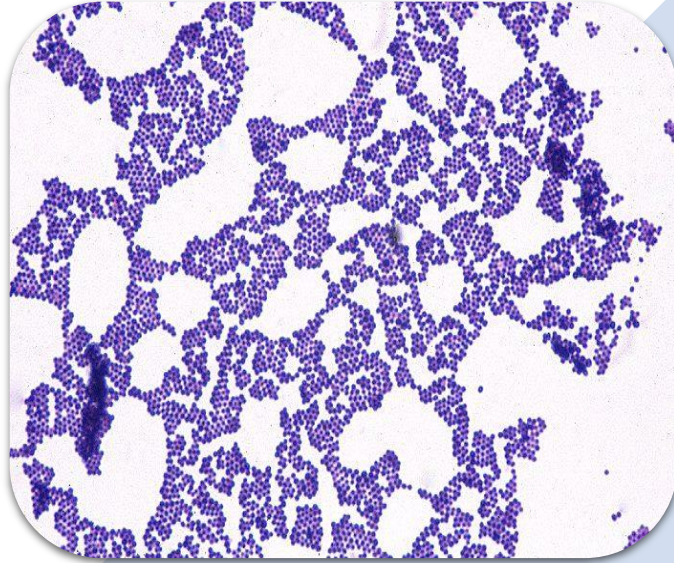


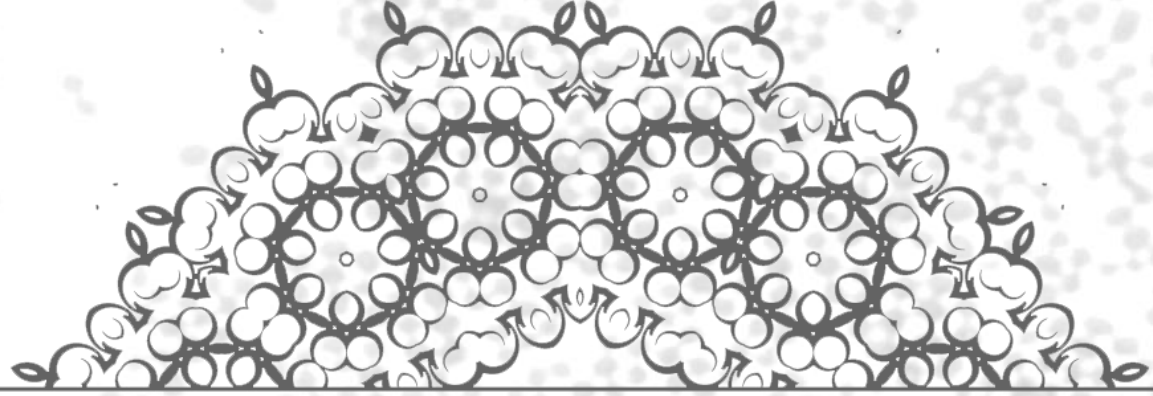
# Gram Positive Group











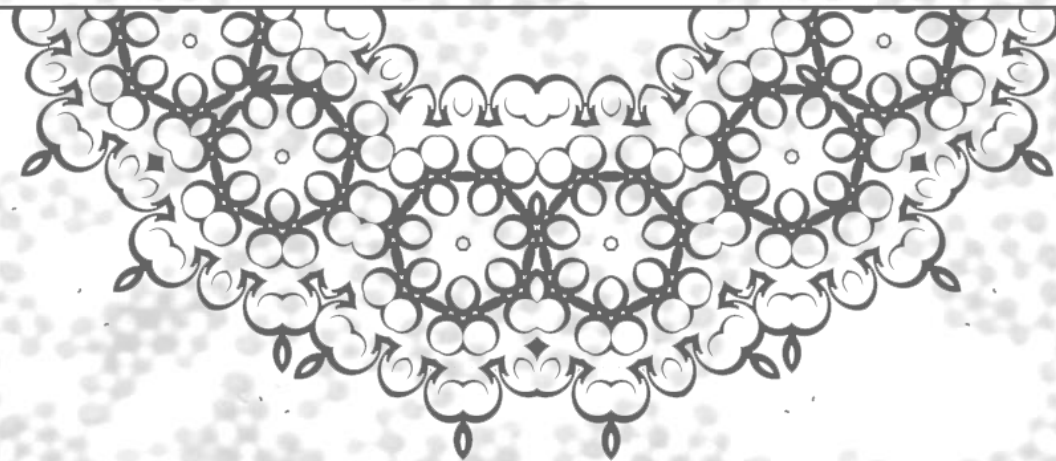
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# Lab 1

## Gram Positive Group

### ( Cocci – Staphylococci )

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## ❖ General Characters of Staphylococci :

● Grape – like (cluster), 0.5 – 1.5  $\mu\text{m}$  in diameter.

● 4 Non (Motile – Spore forming – capsulated – fastidious).

● Facultative anaerobic.

● Fermentative.

● Catalase positive.



## ❖ Characters of *Staphylococcus aureus* :

Production of coagulase, phosphatase and DNase.

Ferment Mannitol.

Gelatin liquefied.

B-hemolysis on blood agar.

Acidification & clotting of litmus milk.



## Three species of staphylococci have medical importance :

	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>
Pathogenicity	Pathogenic	Non pathogenic	Pathogenic Cause UTI in female
Distribution	Nose (nares)	Nares & skin	Skin

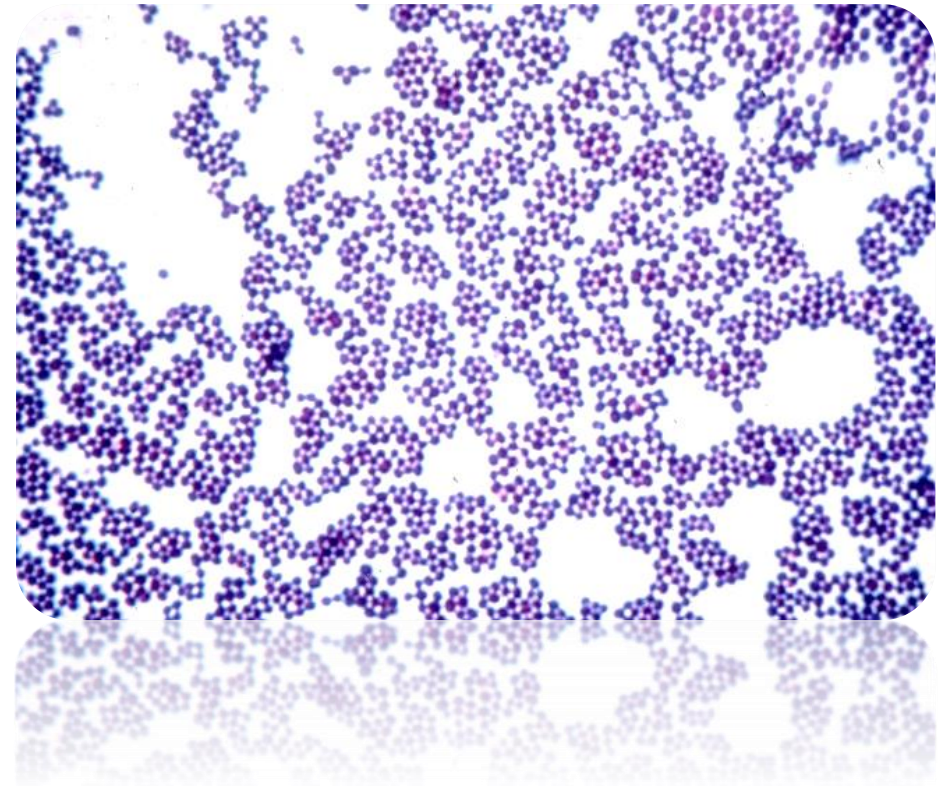
## ❖ Laboratory diagnosis of *Staphylococcus* sp. :

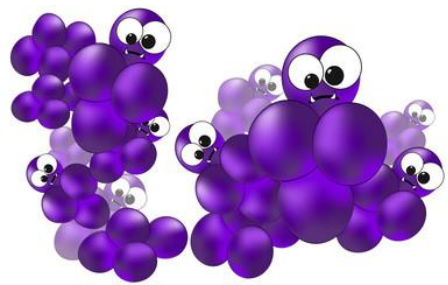
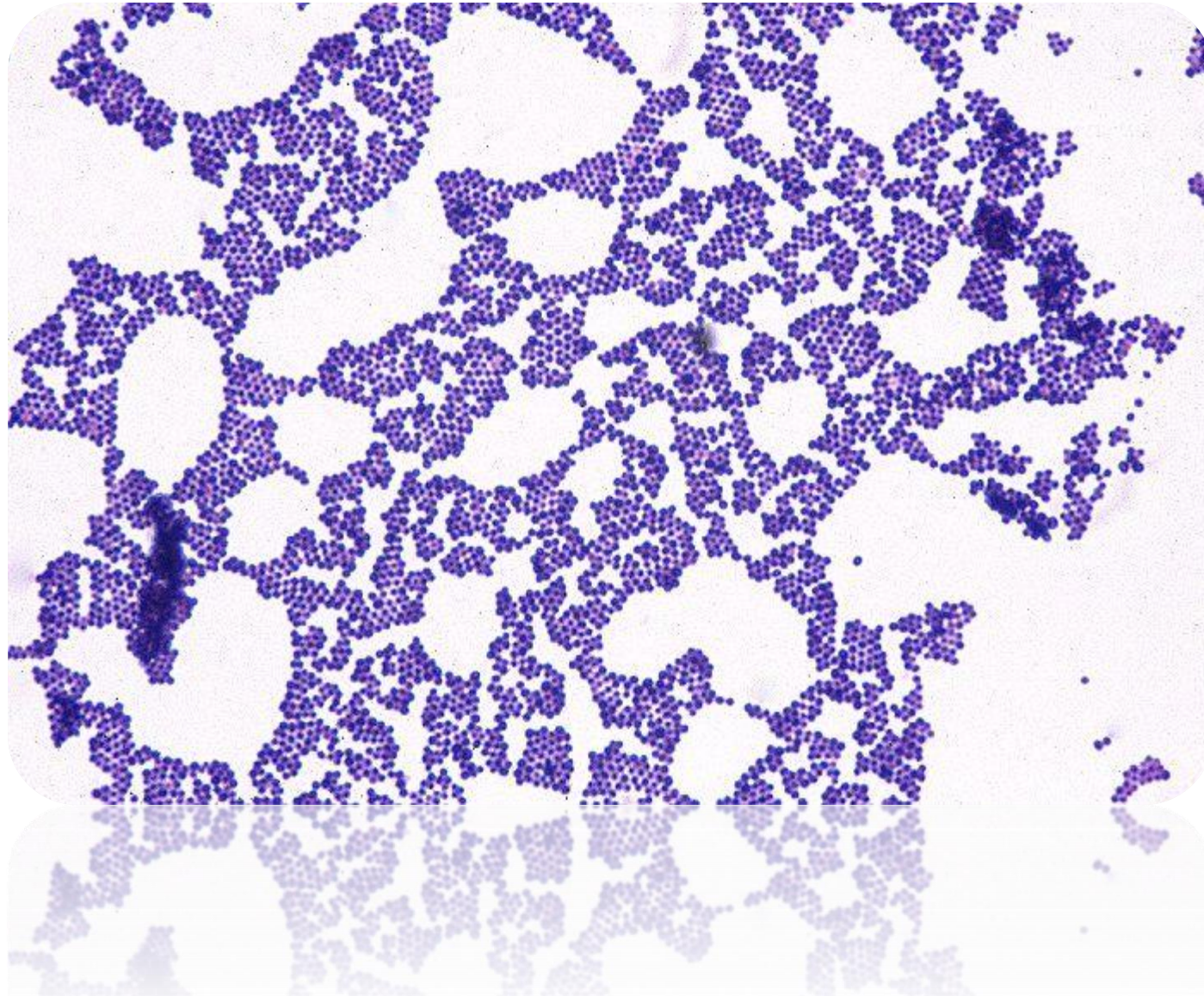
### ■ Specimen :

Pus, urine, stool, blood and CSF.

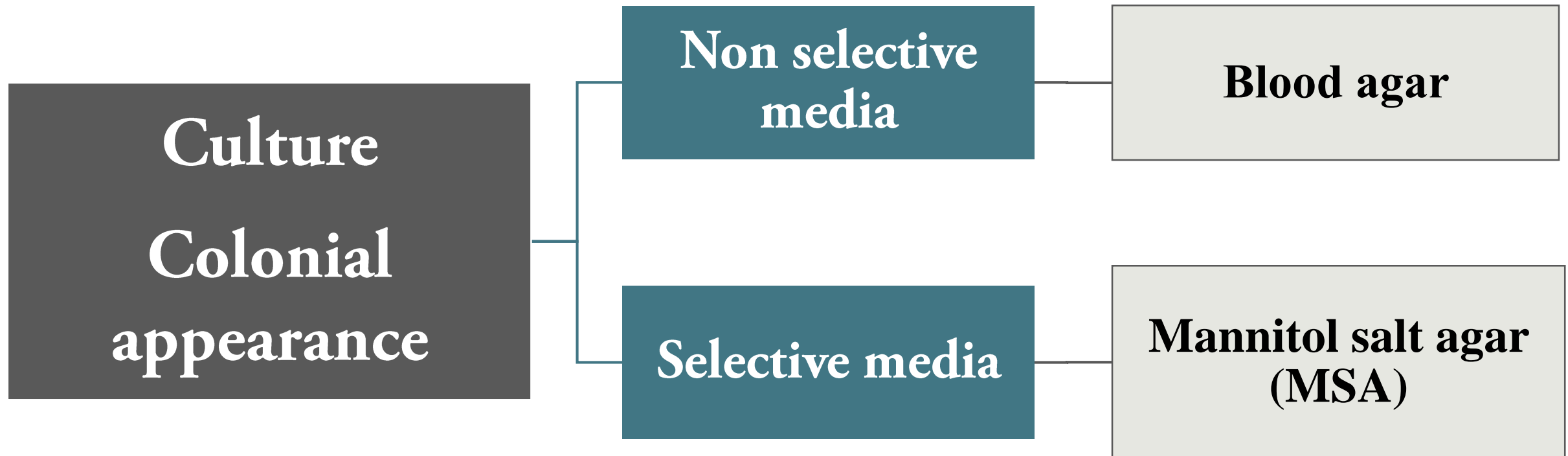
### ■ Gram Stain :

Gram positive cocci arranged in cluster.









## Non Selective Media (Blood Agar)

	<i>S. aureus</i>	<b>Other <i>staphylococci</i></b>
<b>Color</b>	Pigmented	Unpigmented
<b>Morphology</b>	Smooth & entire	<ul style="list-style-type: none"> <li>▪ Smooth, entire, opaque.</li> <li>▪ Glistening.</li> <li>▪ Slightly raised to convex</li> </ul>
<b>Hemolysis</b>	Hemolytic	Non hemolytic



## Selective Media (MSA)

**MSA is selective and differential media because :**

### **Selectivity**

The cause of selectivity due to presence of high salt concentration, NaCl (7.5%)

### **Differentially**

The cause of differential because contains mannitol (sugar) and phenol red (pH indicators turns yellow in acidic pH and turns red in alkaline pH).

## Results on MSA

	<i>S. aureus</i>	<b>Other <i>staphylococci</i></b>
<b>Ferment Mannitol</b>	Positive	Negative
<b>Color of colonies</b>	Yellow	Red
MSA is used for distinguished pathogenic staphylococci from non pathogenic staphylococci.		



There are 4 test to  
differentiate between  
*Staphylococci* species

**Catalase test**

**Coagulase test**

**DNase test**

**Novobiocin  
sensitivity**

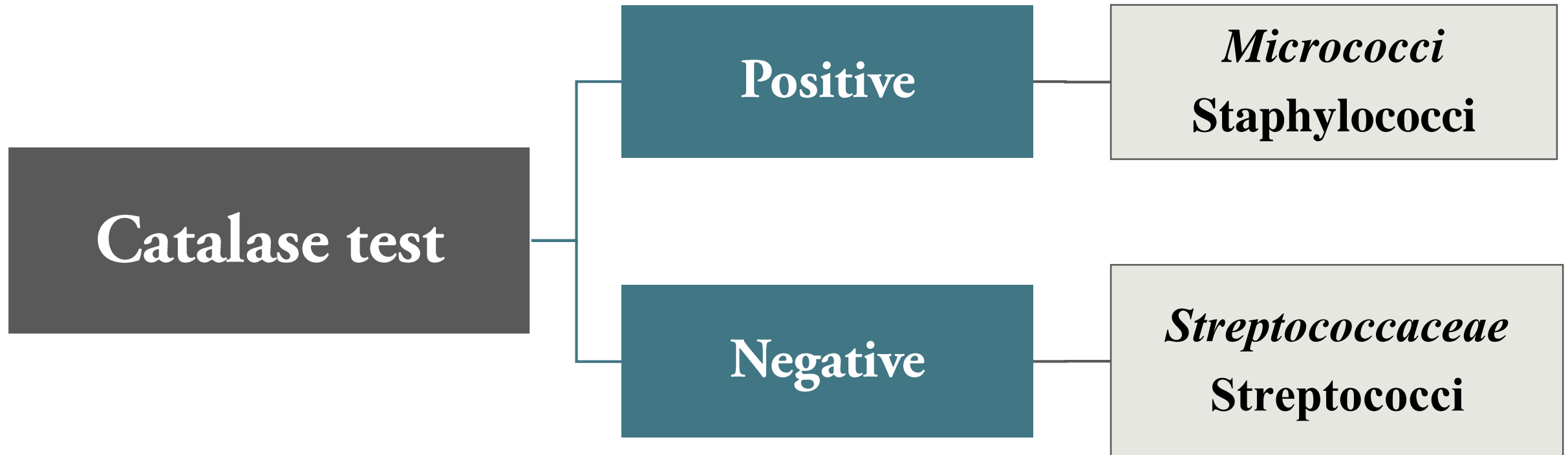
## ❖ 1<sup>st</sup> : Catalase Test :

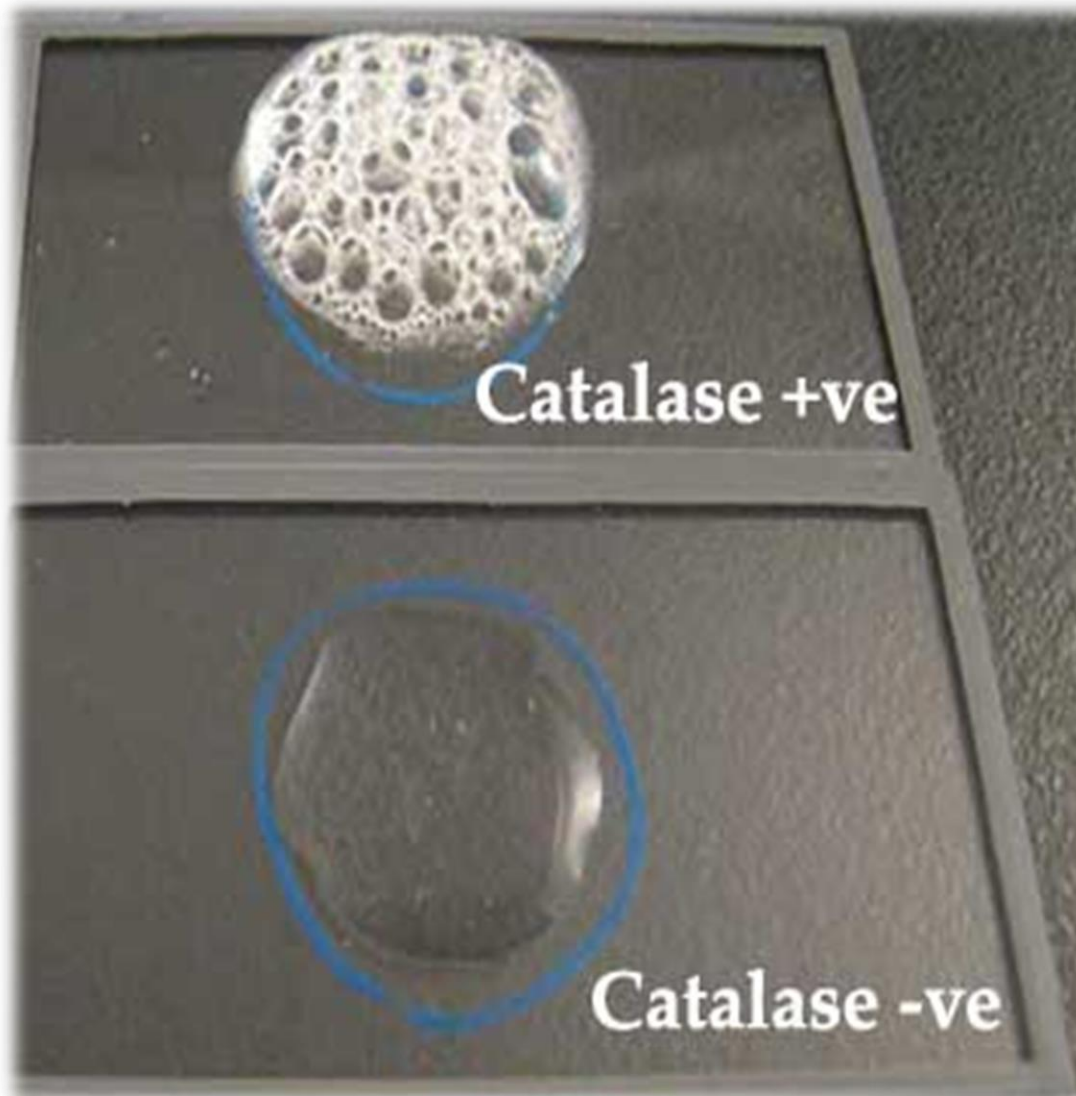
- The catalase test is distinguished *streptococci* from *staphylococci* and *Micrococci*.

### • Procedure :

1. Flood culture with drops of 3% H<sub>2</sub>O<sub>2</sub>.
  2. Catalase-positive cultures bubble at once.
- The test should not be done on blood agar because blood itself will produce bubbles

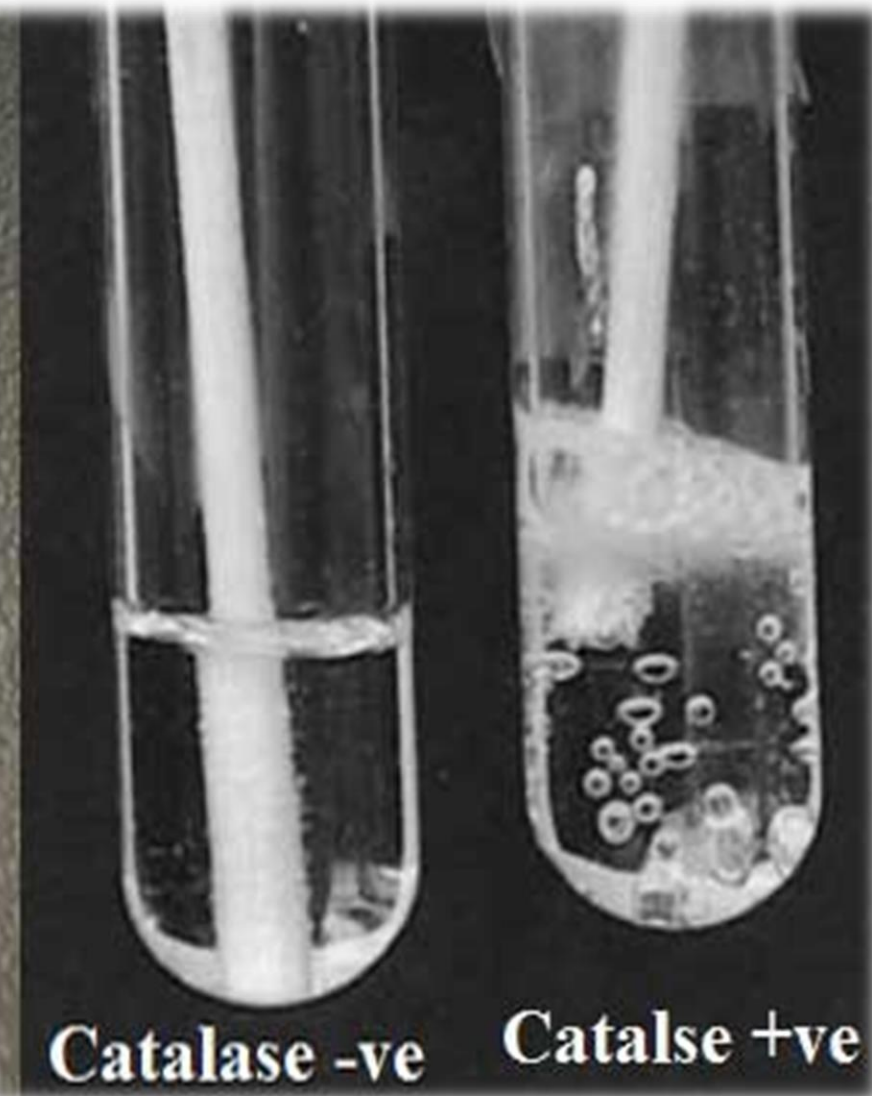






**Catalase +ve**

**Catalase -ve**



**Catalase -ve**

**Catalase +ve**

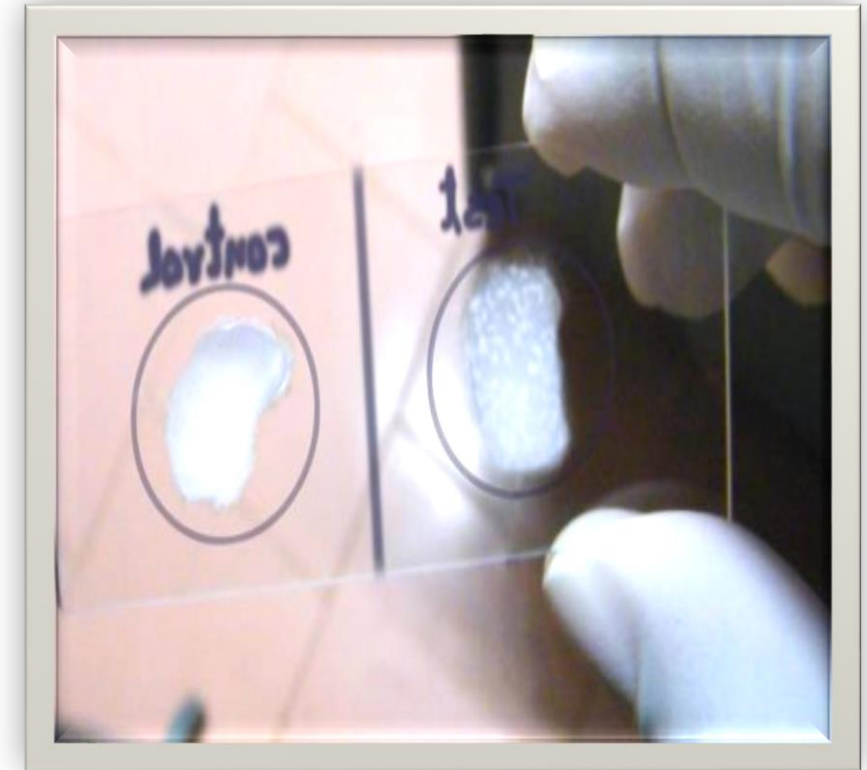


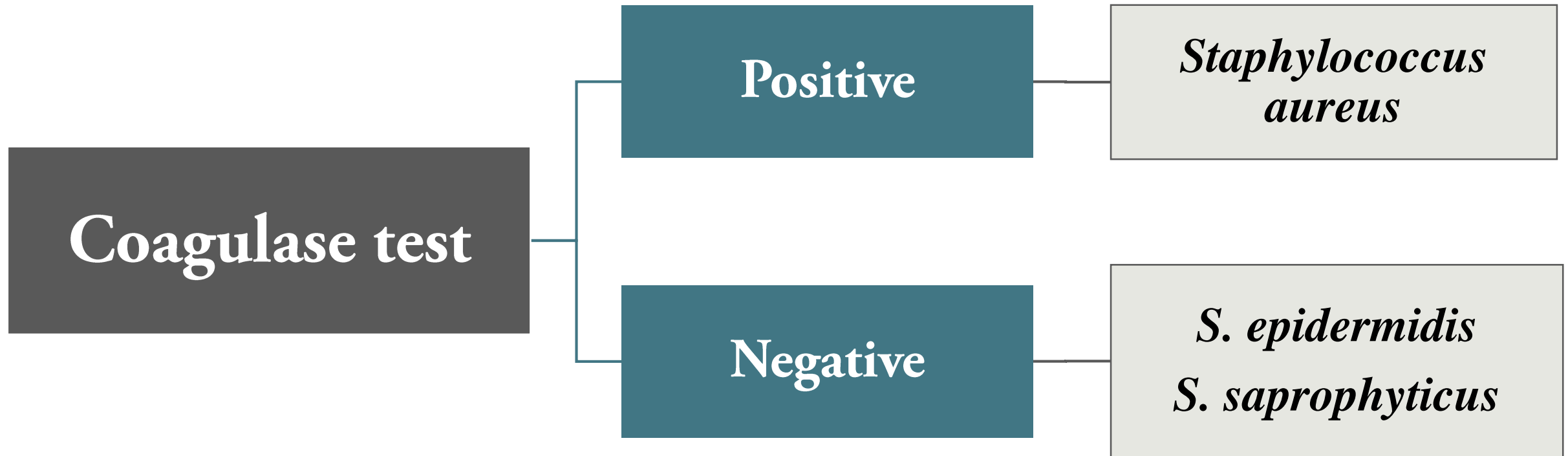


## ❖ 2<sup>nd</sup> : Coagulase Test :

### • Principle :

- This test used to differentiate between *S. aureus* & other *Staphylococcus* species.







# There are two methods for Coagulase test

Tube method



Slide method



# Compare between Coagulase methods

	Tube Method	Slide Method
<b>Procedure</b>	<ul style="list-style-type: none"> <li>▪ Mix 0.1 ml of culture + 0.5 ml of plasma.</li> <li>▪ Incubate at 37 °C for 4 h.</li> <li>▪ Observing the tube for clot formation.</li> <li>▪ Any degree of clotting constitutes a positive test.</li> </ul>	<ul style="list-style-type: none"> <li>▪ Used to detect bound coagulase or clumping factor.</li> <li>▪ Add one drop heavy bacterial suspension and one drop of plasma on clean slide.</li> <li>▪ Mixing well and observing for clumping within 10 seconds.</li> </ul>
<b>Advantage</b>	More accurate	Rapid diagnosis
<b>Disadvantage</b>	Time consumed	Less accurate



## ❖ 3<sup>rd</sup> : Deoxyribonuclease Test (DNase) :

### • Principle :

- DNA is composed of two chains of repeating nucleotides.
- DNA is insoluble in acid, while nucleotides soluble in acid.
- DNA is hydrolyzed into oligonucleotides by the action of DNase

## DNase test

### Procedure

- Inoculate DNA agar with tested organism in circular motion.
- Incubate at 37 °C for 24 – 48 H.
- Observe DNase activity by adding 1N HCl to the agar surface.

### Result

- Present of clearing zone indicates as a positive reaction.
- The zone represents the absence of DNA.
- The medium around colonies not producing DNase remains opaque, which is a reflection of the precipitation of DNA by the added acid.



# DNase test

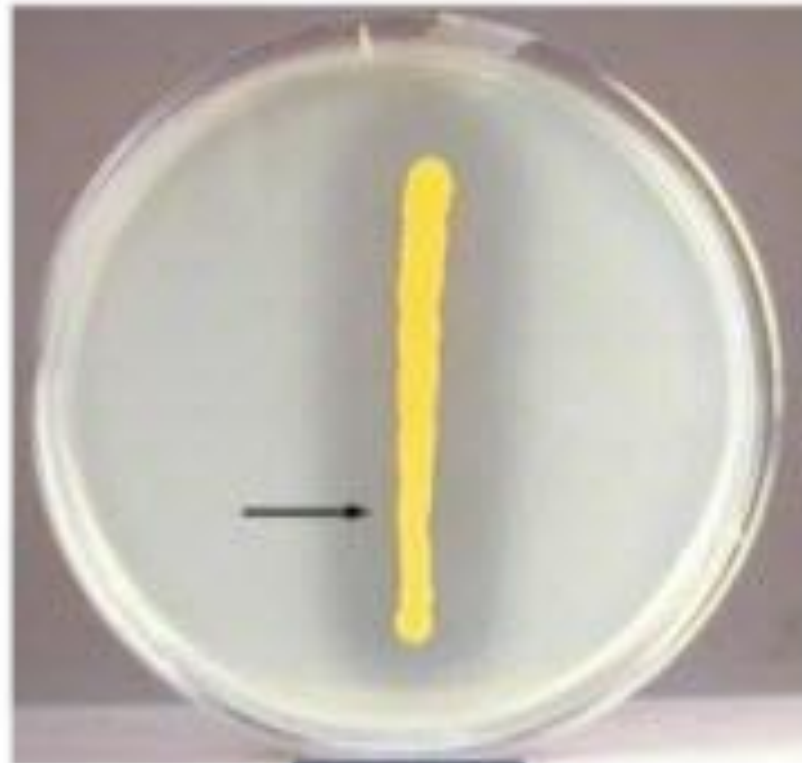
Positive

*Staphylococcus aureus*

Negative

*S. epidermidis*  
*S. saprophyticus*





+



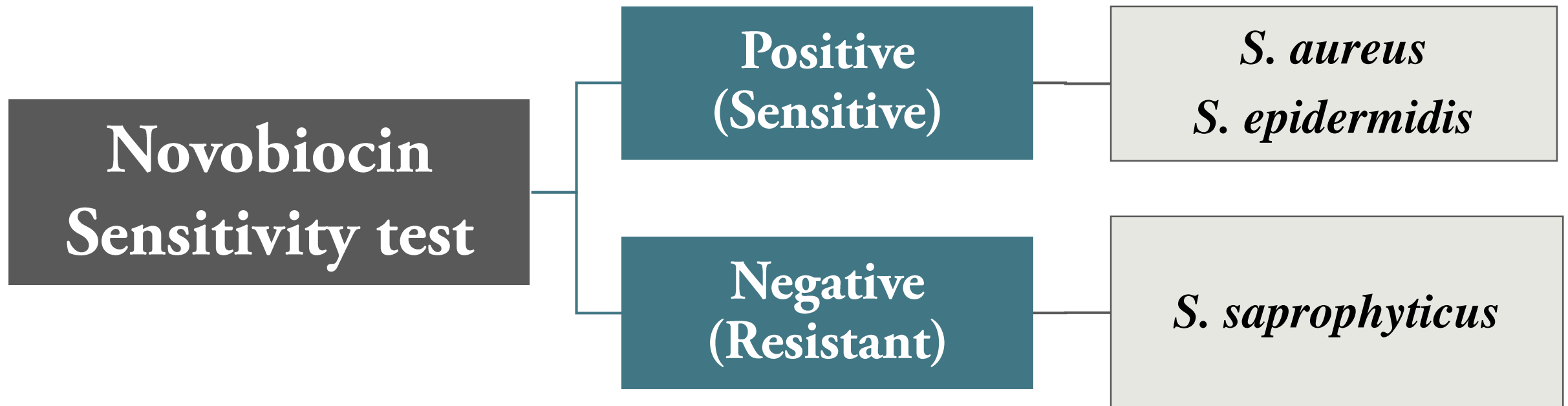
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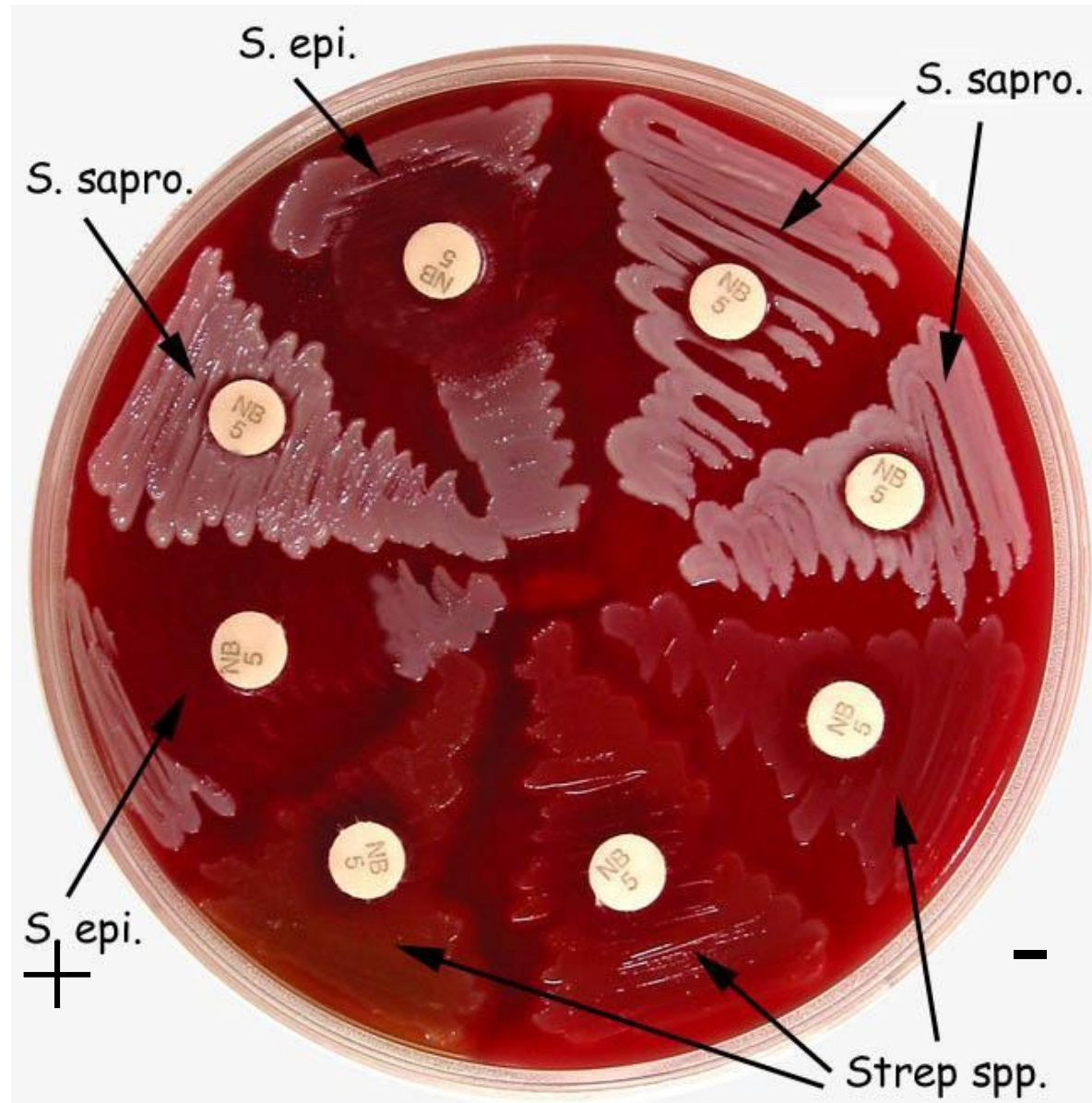
## ❖ 4<sup>th</sup> : Novobiocin Sensitivity :

- A simple disk diffusion test for estimating Novobiocin susceptibility used to distinguish *S. saprophyticus* from other clinically species

### • Procedure :

- Inoculated overnight culture on Mueller-Hinton agar.
- Add Novobiocin disk on inoculated plate.
- Incubate at 37 °C overnight.







# Any Questions

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