

# THROAT SWAB

**Gram Positive  
Coccus**

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**Staphylococcus  
Spp.**

**Streptococcus  
Spp.**

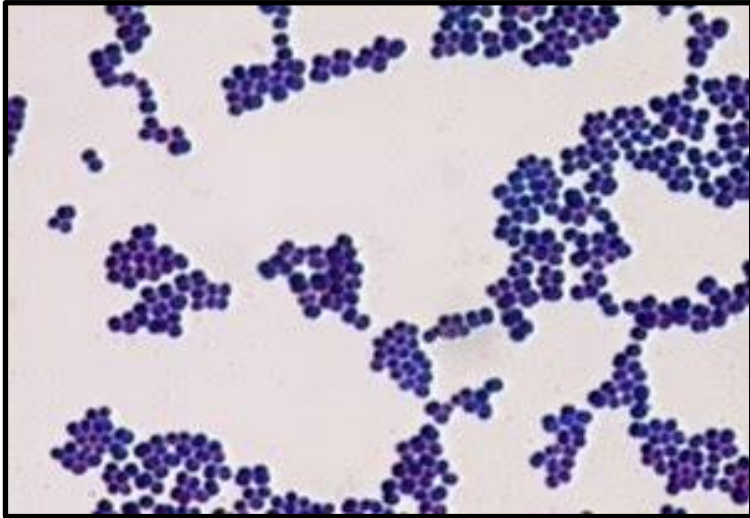
**- Our lab today will be about common gram positive bacteria that may cause throat infection and go in details how to differentiate between the species.**

**-A THROAT SWAP culture or throat culture is a test commonly used to diagnostic bacterial infections in the throat.**

**-The most common gram positive Cocci bacteria are staphylococcus species and streptococcus species beside other bacteria.**

# GRAM STAIN

## Staphylococcus

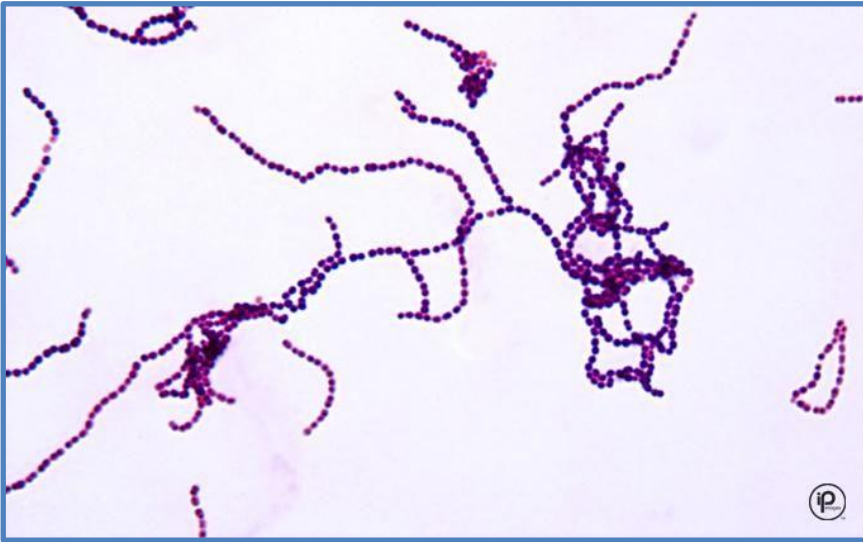


In the picture above ,the **staphylococcus** are gram –positive cocci arranged in a grapes –like clusters.

-Staphylococcus includes at least 40 species ,the most common species is that associated with clinical infection are : 1)staphylococcus aureus ,2)staph epidermidis ,3)staph hemolyticus,4)staph hominins ,5)and staphylococcus albus.

# GRAM STAIN

## Streptococcus •



In the picture above, **Streptococcus** species are gram –positive cocci arranged in a chain or diplococcus.

A- Staphylococcus .albus

B- Staphylococcus.aureus

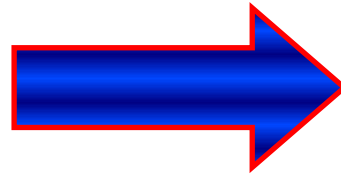
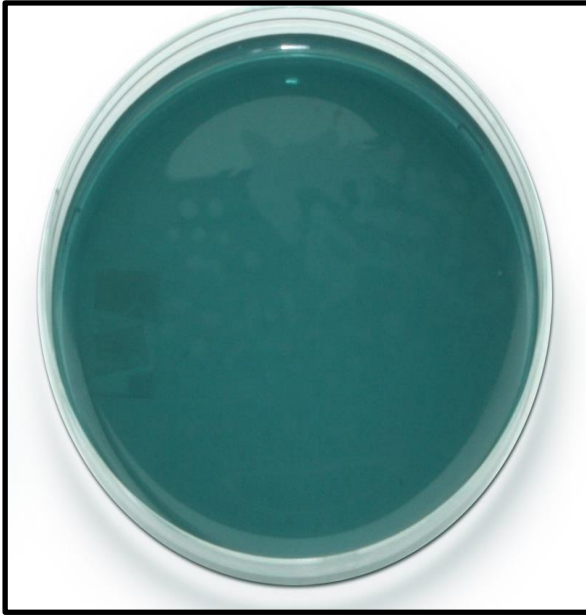


Blood agar

- **Staphylococcus aureus** appears yellow golden or yellow colonies often with hemolysis when grown on blood agar plates, the golden appearance is the etymological root of the bacterium's name "aureus" which means golden in Latin. While other staphylococcus species such as **albus orepidemidis** appear as white colonies on blood agar.

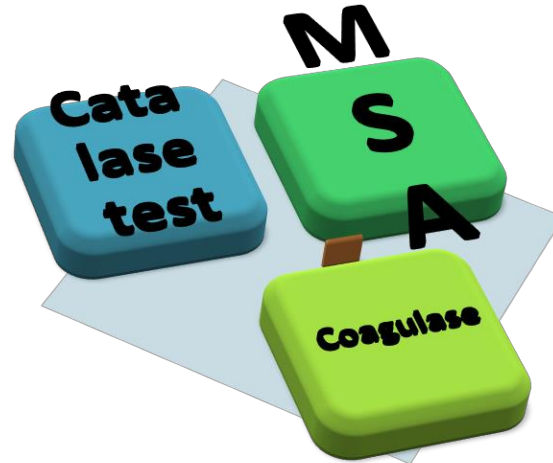


CLED media  
Lactose fermentation ( +ve )  
staphylococcus spp.



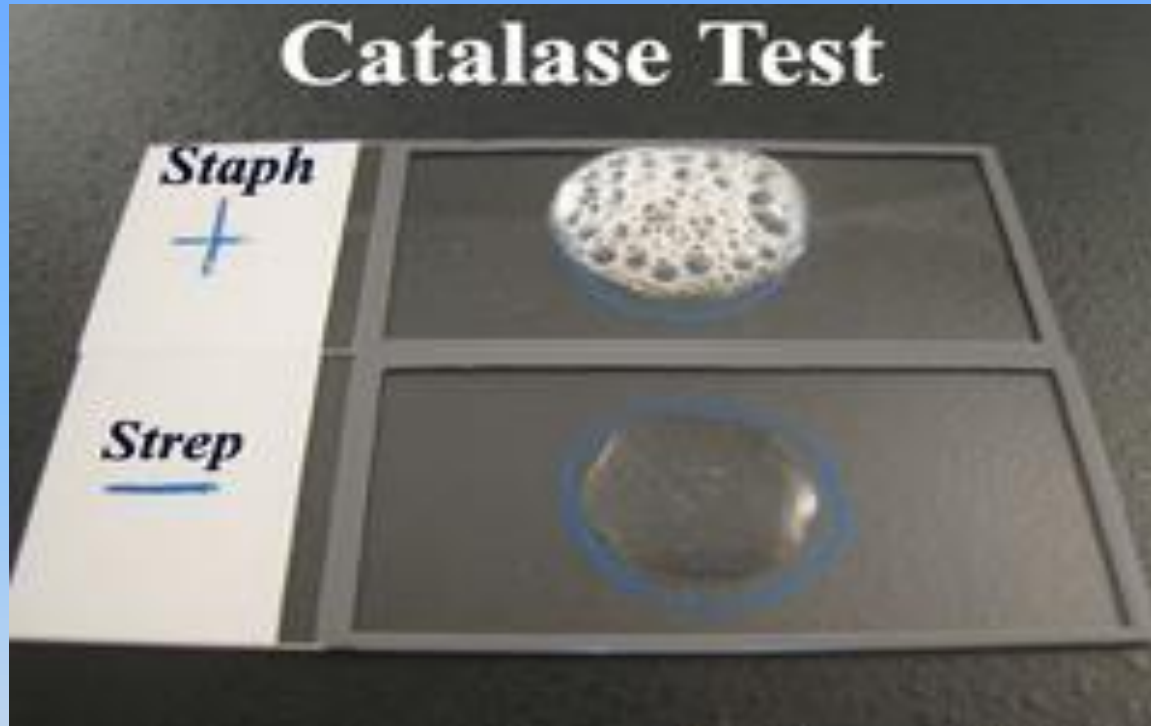
Staphylococcus species and specially **staphylococcus aureus** grow on the surface of CLED media and show dark yellow colonies due to its ability to ferment lactose.

# Test for differentiation of Staphylococcus species



The major test reaction that should be used to differentiate between staphylococcus and streptococcus is **catalase test**, while the major test reaction that should be used to differentiate between staphylococcus aureus and other staphylococcus species such as albus or others, we have to use **MSA (mannitol salt agar) and coagulase tests.**

# (1)Catalase test



This test is used to identify organisms that produce the enzyme catalase, this enzyme detoxifies hydrogen peroxide by breaking it down to water and oxygen gas , the bubbles resulting from production of oxygen gas clearly indicate a catalase-positive result. Staphylococcus gives a catalase-positive result, while streptococcus gives a catalase-negative result.



# (2)MSA

## Mannitol salt agar media

**MSA:- mannitol salt agar 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).**

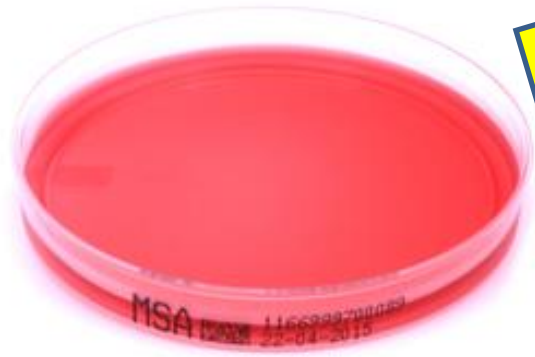
**MSA also contains the sugar mannitol and the PH indicator phenol red. If an organism can ferment mannitol, an acidic by-product is formed that will cause the phenol red in the agar to turn yellow.**

**Staphylococcus aureus will ferment mannitol, so it will turn the media color to yellow, while other staphylococcus species such as staphylococcus albus and staphylococcus epidermidis will not ferment mannitol, and the media will remain red in color.**

**See the pictures in the next slide....**

## (2)MSA

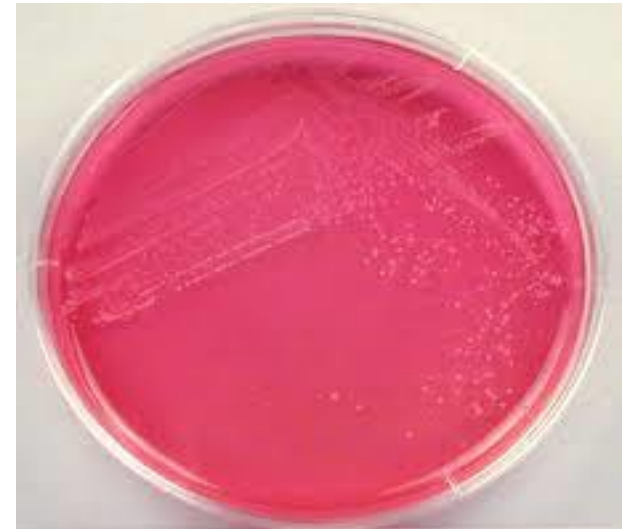
# Mannitol salt agar media



**S.aureus**



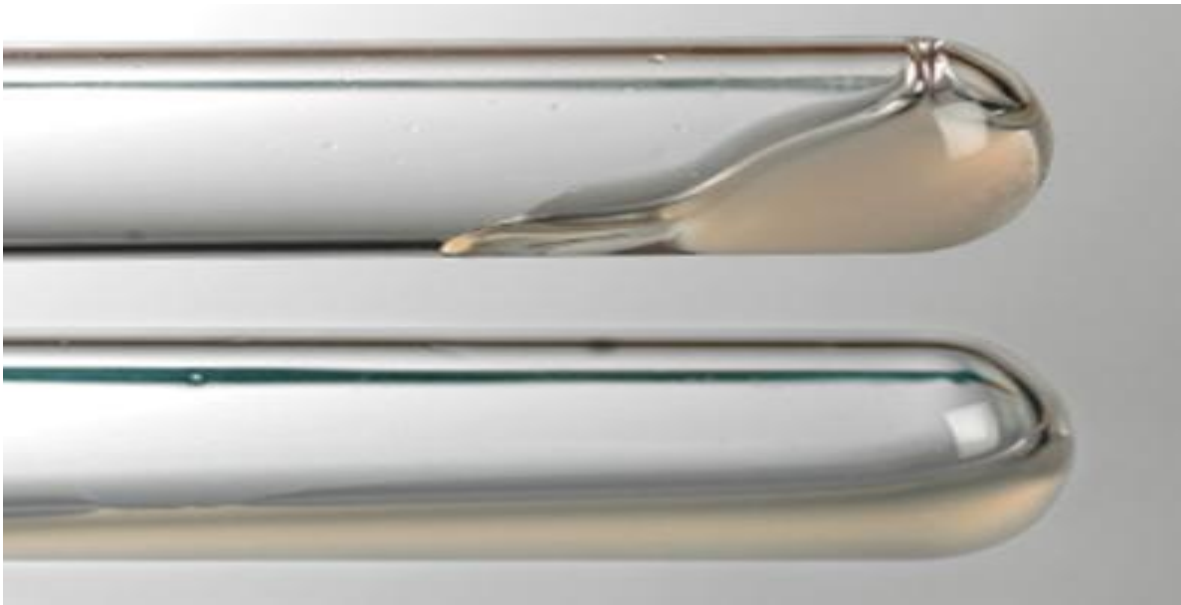
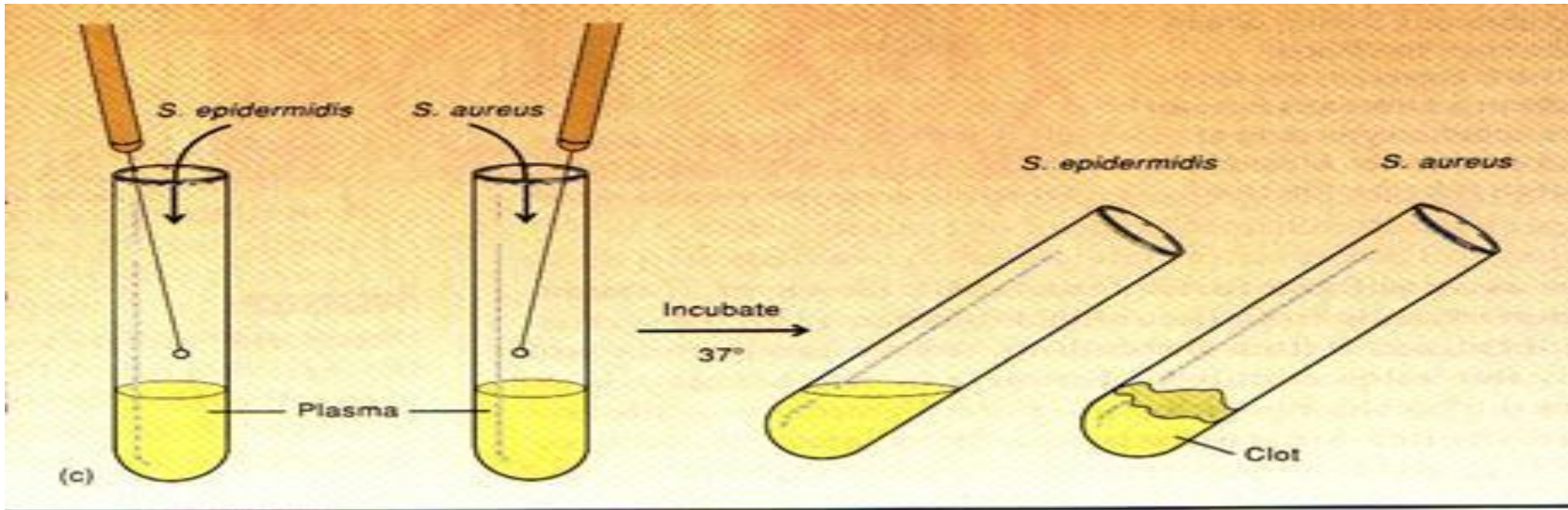
**S.albus**



## **(3) Coagulase test**

- **used to differentiate staphylococcus aureus (coagulase positive) from other staphylococcus species (coagulase negative).**
- **- coagulase is an enzyme produced by staphylococcus aureus that converts soluble fibrinogen in plasma to insoluble fibrin.**
- 
- **test method:**
- **1- suspension of an organism is suspended and incubated with plasma at 37o C in a tube, clot formation within 4 hours indicates a positive test and this indicates staph aureus.**
- **2- leave negative tubes at room temperature overnight and re-examine, this step is essential for some strains of staphylococcus aureus (including many amersa) that produce a delayed clot which is rapidly lysed at 37o C by the organism staphylokinase.**

# Coagulase test



**Streptococcus** are Gram-positive aerobic organisms that cause many disorders including pharyngitis, pneumonia, wound and skin infection, sepsis, and endocarditis.

Three different types of streptococcus are initially differentiated by their appearance when they are grown on sheep blood agar:

1) The first group is the  $\beta$ -hemolytic streptococcus: produce zones of clear, complete hemolysis around each colony such as group A streptococcus pyogenes and group B streptococcus agalactiae.

2)  $\alpha$ -hemolytic streptococcus: such as streptococcus pneumonia and streptococcus viridans, they are surrounded by green discoloration resulting from incomplete hemolysis.

3)  $\gamma$ -hemolytic: non hemolytic such as enterococcus species as Enterococcus faecalis .

See the next slide

# Streptococcus

**$\alpha$ -hemolytic**

green,  
partial hemolysis

pneumoniae

optochin sensitive,  
bile soluble,  
capsule =>  
quellung +

Viridans

mutans, sanguis  
optochin resistant,  
not bile soluble,  
no capsule

**$\beta$ -hemolytic**

clear,  
complete hemolysis

pyogenes

Group A,  
bacitracin sensitive

agalactiae

Group B,  
bacitracin resistant

**$\gamma$ -hemolytic**

no hemolysis

Enterococcus

E. faecalis,  
E. faecium

# Hemolysis on sheep blood agar

## Blood Agar:

Shows three types of hemolysis

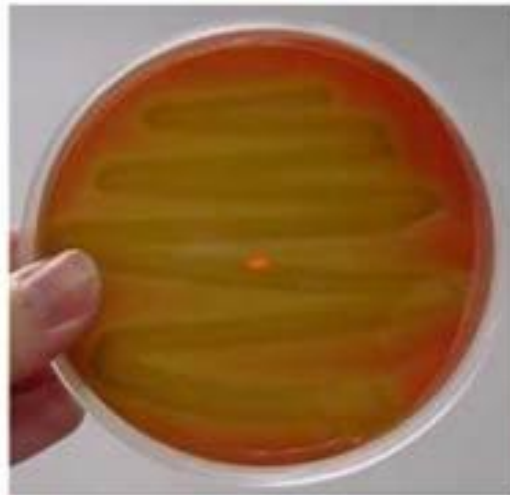
$\alpha$  Hemolysis

$\beta$  Hemolysis

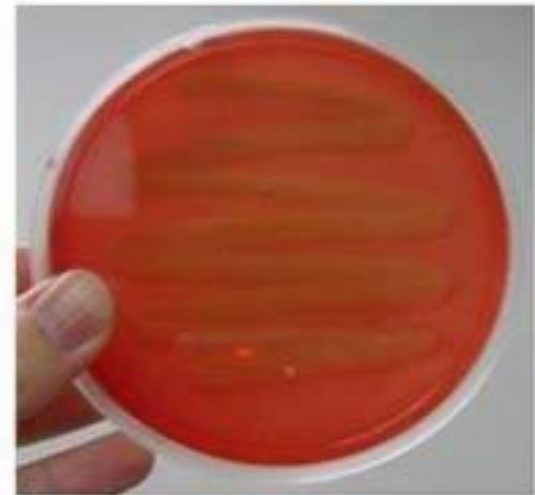
$\gamma$  Hemolysis



**Beta Hemolysis**



**Alpha Hemolysis**

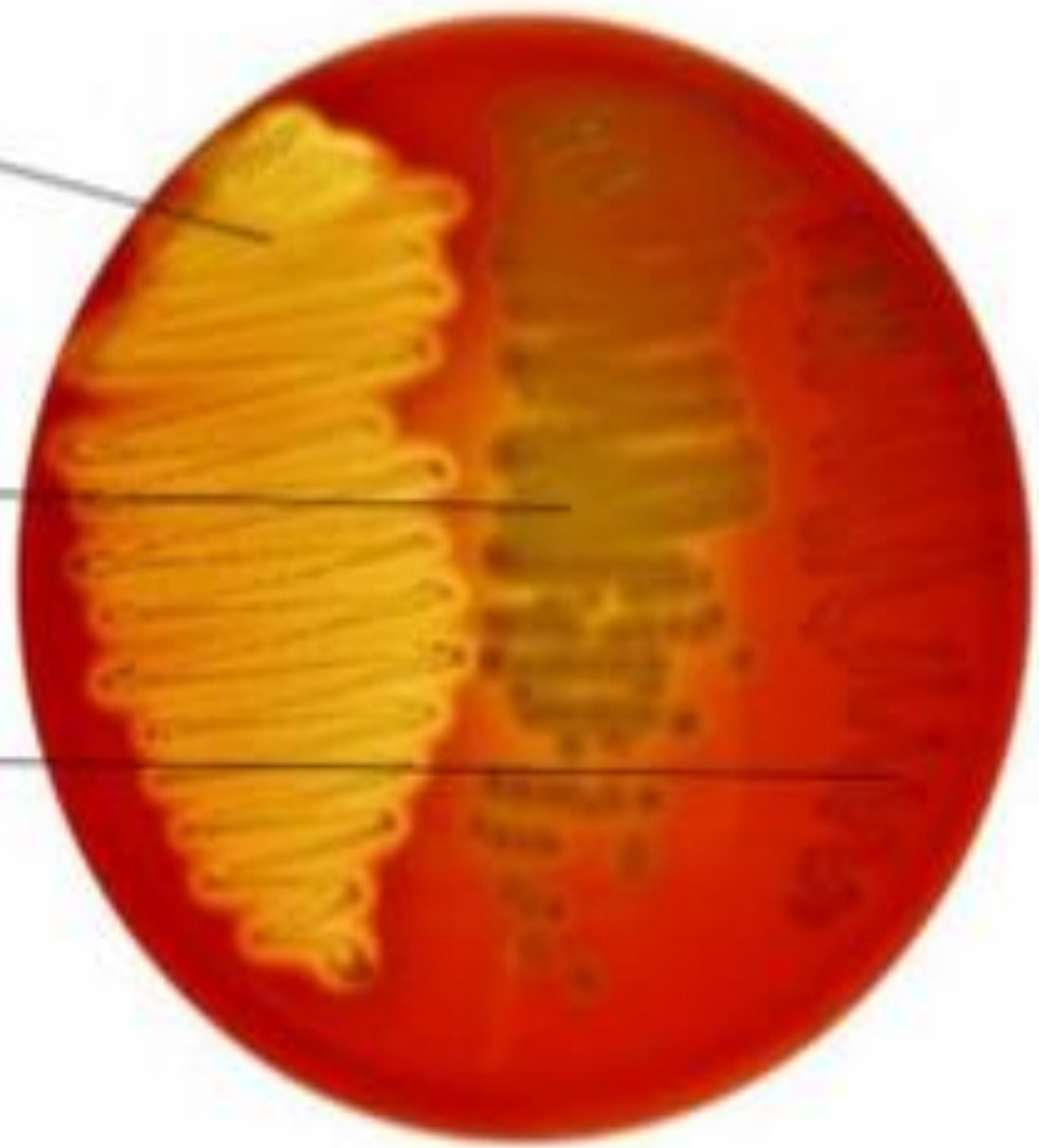


**Gamma Hemolysis**

**Beta**

**Alpha**

**None**





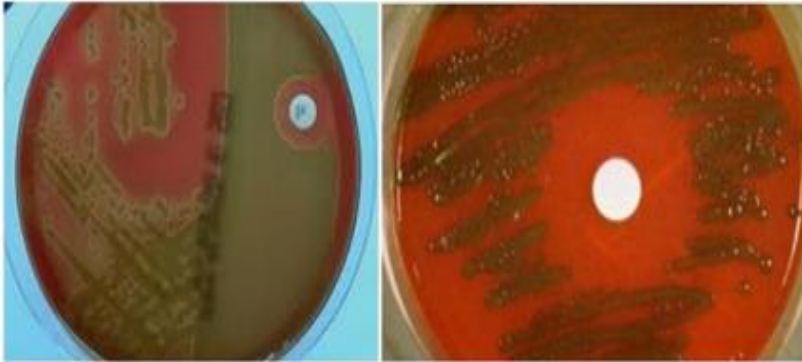
## Differentiation between $\alpha$ -hemolytic streptococci

	Hemolysis	Optochin sensitivity
<i>S. pneumoniae</i>	$\alpha$	Sensitive ( $\geq 14$ mm)
<i>Viridans strep</i>	$\alpha$	Resistant ( $\leq 13$ mm)

- Streptococcus pneumoniae can be differentiated from Streptococcus viridans using an optochin test, as ***Streptococcus pneumoniae is optochin-sensitive*** due to its specific membrane composition and ATPase activity, while ***Streptococcus viridans is optochin-resistant***.
- Streptococcus pneumoniae possesses a polysaccharide capsule that serves as a major virulence factor.
- **Note:** Lancefield antigens are not used to classify these bacteria, as both are  $\alpha$ -hemolytic streptococci.

## Optochin test

### *Streptococcus pneumoniae*



*Streptococcus pneumoniae* strain on blood agar showing alpha hemolysis (green zone surrounding colonies). Note the zone of inhibition around a filter paper disc impregnated with optochin. (sensitive to optochin)

This pic reveals strep pneumonia since the growth of bacteria that are optochin sensitive will show a zone of inhibition around an optochin disk

while the growth of bacteria that are optochin resistant will not be affected as it is shown in this pic which represents strep viridans.

## Optochin test

### *Streptococcus viridans*



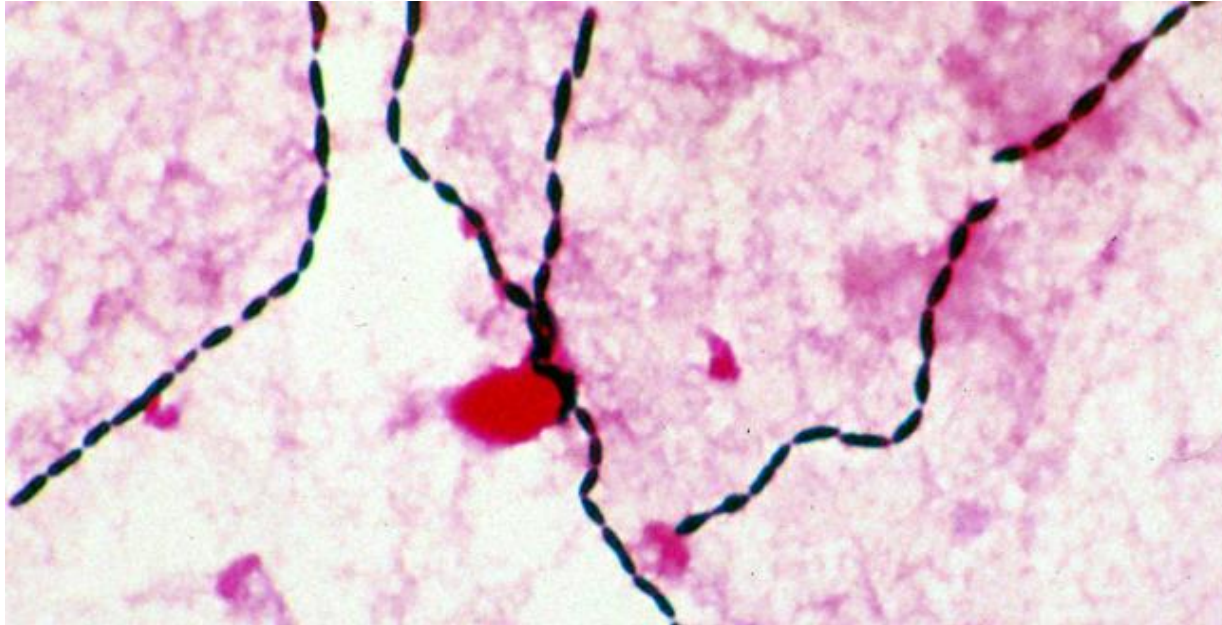
*Streptococcus viridans* strain on blood agar showing alpha hemolysis (green zone surrounding colonies). No zone of growth inhibition (Resistant) around a filter paper disc impregnated with optochin.

# Streptococcus pneumoniae



**This is strep pneumonia cells under light microscope, it is gram+ lancet shaped coccus elongated with a slightly pointed outer curvature, usually they are seen as pairs of coccus or diplococcus but they may also occur in single and in short chains.**

# Streptococcus viridans



**This slide represents strep viridans under light microscope, it is gram+ coccus and usually forms short to long chains and are often elongated.**

# bacitracin susceptibility test

Differentiation between $\beta$ -hemolytic streptococci		
	Hemolysis	Bacitracin sensitivity
<i>S. pyogenes</i>	$\beta$	Susceptible
<i>S. agalactiae</i>	$\beta$	Resistant

**bacitracin susceptibility test:-** used to distinguish group A strep: strepto pyogenes from other streptococcus such as group B streptococcus agalactiae.

**when grown on blood agar group A streptococcus is sensitive to bacitracin that will show a zone of inhibition around the bacitracin disk, while strep agalactiae that is resistant to bacitracin, growth will not be affected and won't show a zone of inhibition growth.**



**The right blood media represents strep-pyogenes since it's sensitive to bacitracin while the blood agar on the left represents strep agalactiae which is resistant to bacitracin disk.**

# **Gamma hemolysis streptococcus**



**Enterococcus  
Group D  
- E.feacalis**

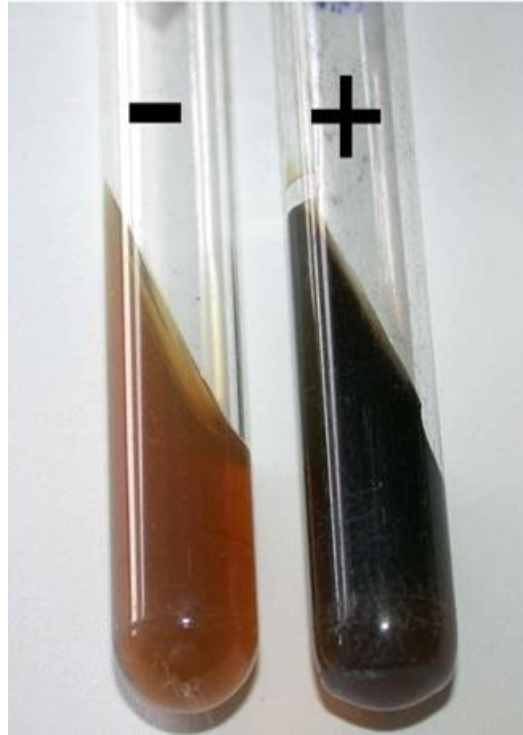


**Other than  
Enterococcus group D**

-gamma hemolysis is indicative of no hemolysis or lack of hemolysis around the bacterial colony.

-An example of non-hemolytic streptococcus are entero-coccus group D such as streptococcus feacalis and non-enterococcus or other than enterococcus group D.

## *Bile-Esculin*



Other than  
Enterococcus  
Group D  
**Negative**

**Enterococcus  
Group D  
Positive**

-Bile-Esculin test is used to differentiate between enterococcus group D and other non-enterococcus species.

-Enterococcus group D give positive bile esculin test.

-What is bile esculin test? It is based on the hydrolysis of Esculin into glucose and esculitin by a microorganism that produces the enzyme esculinase.

-esculitin reacts with iron salt (ferric citrate) in the medium to form a phenolic iron complex which produces a dark brown or black color.



Thank you for listening, any  
questions?



**THE END**