

# Gram positive cocci

Opto 435

# Gram positive Cocci

## Catalase test

+ve result  
Staphylococci

-ve result  
Streptococci

## Coagulase test

## Haemolysis on blood agar

+ve result

*S. aureus*

-ve result

## Novobiocin disk

Susceptible

*S. epidermidis*

Resistant

*S. saprophyticus*

alpha  
haemolysis

## Optechin test

Susceptible

*S. pneumonia*

Resistant

*S. viridans*

beta  
haemolysis

## Bacitracin disk

Susceptible

*S. piogen*  
(*S. group A*)

Resistant

*S. group B*

gamma (no)  
haemolysis

growth

in high salt  
concentration

NaCl

growth

in high heat

+ve

Bile esculin  
test

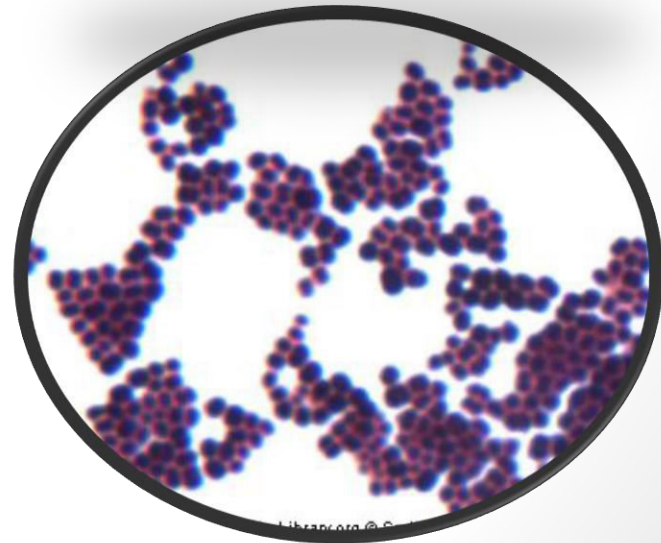
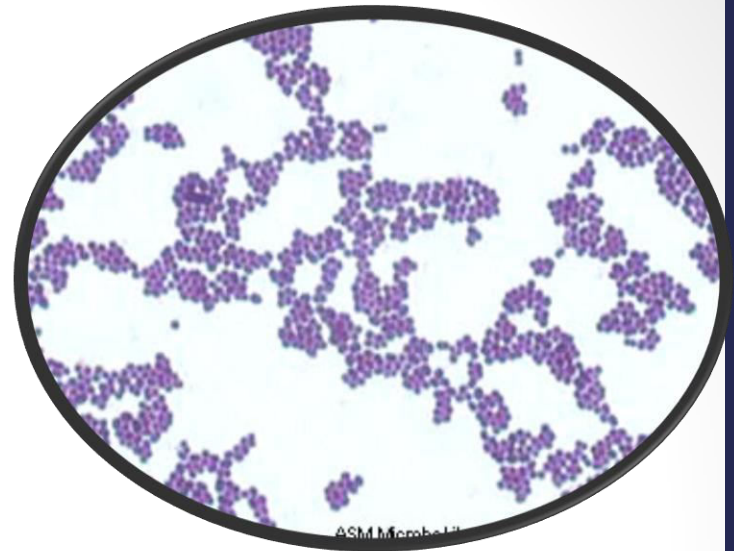
Black color  
esculin hydrolysis

*Enterococcy*

(Group D Strep)

# *Staphylococcus sp.*

- Morphology:
  - Gram positive cocci.
  - In clusters
- Culture:
  - Facultative anaerobes
  - Incubation 37°C x 24 hr



# *Staphylococcus sp.*

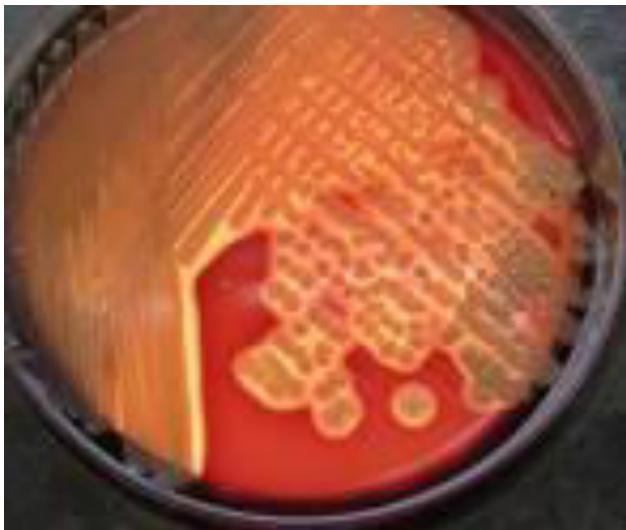
- Coagulase +ve:
  - *Staphylococcus aureus*.
- Coagulase –ve:
  - *Staphylococcus saprophyticus*.
  - *Staphylococcus epidermidis*.

**All Staphylococci are CATALASE +VE**

# CULTURE

**Coagulase +ve**  
***Staphylococcus aureus***

- **Blood Agar:**
  - Creamy colonies 1-2 mm
  - Beta-hemolysis



**Coagulase -ve**  
***Staphylococci***

- **Blood Agar:**
  - Grayish white colonies
  - No hemolysis



# CULTURE

Coagulase +ve  
*Staphylococcus aureus*

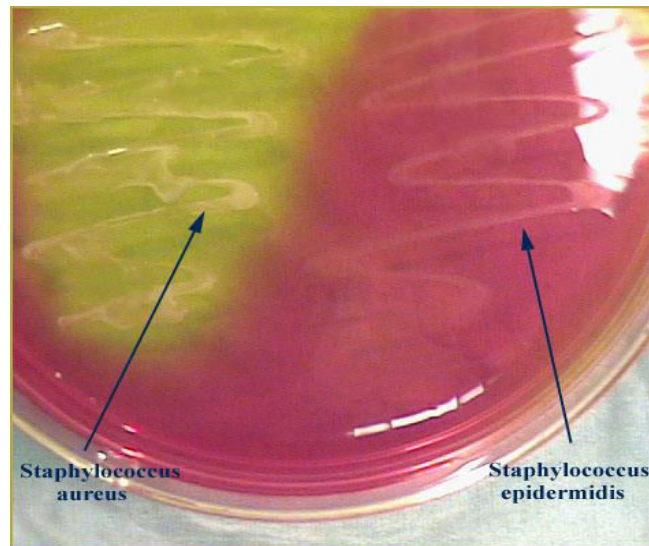
- Mannitol Salt Agar:
  - Growth
  - Change color from Red to Yellow (Mannitol fermenting)

Differential:  
*Staph aureus*  
can ferment  
mannitol and  
give diffused  
yellow color

Coagulase -ve  
*Staphylococci*

- Mannitol Salt Agar:
  - Growth
  - No yellow color no fermentation.

Selective:  
Containing  
very high salt  
conc only  
staph can  
grow



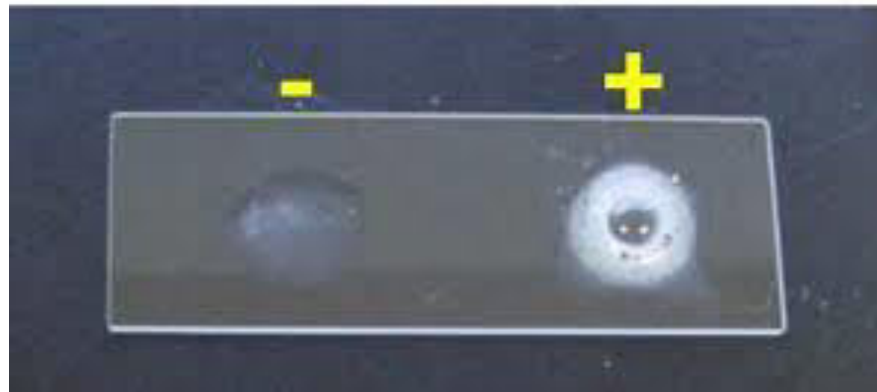
# Mannitol Salt Agar



# Biochemical Tests

## 1. Catalase:

- $2\text{H}_2\text{O}_2 + \text{Catalase enzyme} \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$ 
  - Streptococci vs. Staphylococci



Biochemical Tests



# Coagulase Test (Tube Method)

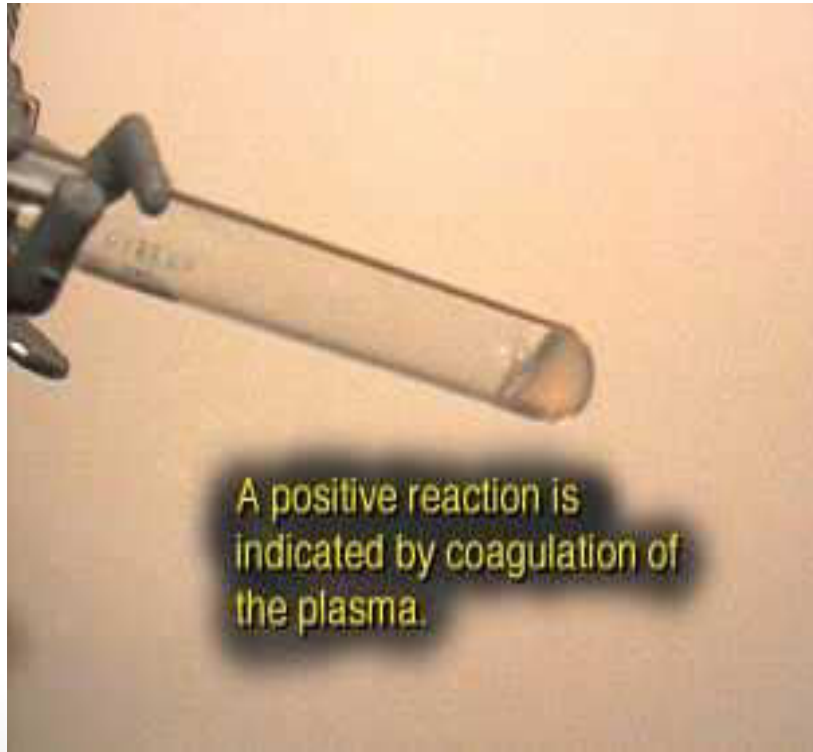
## 2. Coagulase test:

- Fibrinogen in plasma → Fibrin
1. 1 ml pepton + 1 ml EDTA plasma
  2. Emulsify loop full of organism
  3. Incubate 30 min x 37°C
  4. If negative incubate at RT° overnight

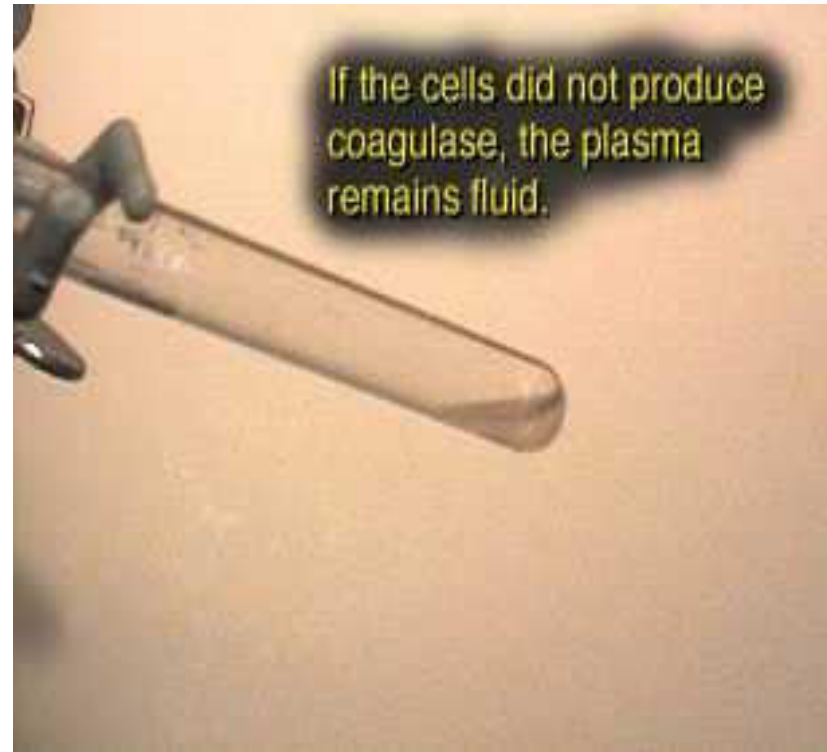


# Coagulase Test

## Coagulase Positive

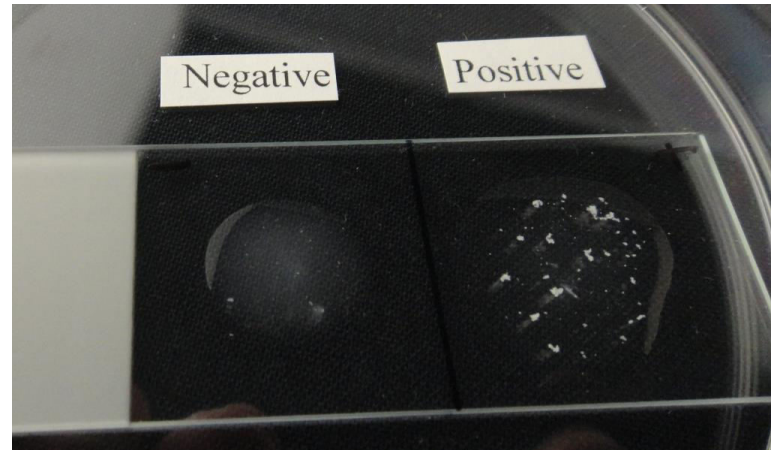


## Coagulase Negative



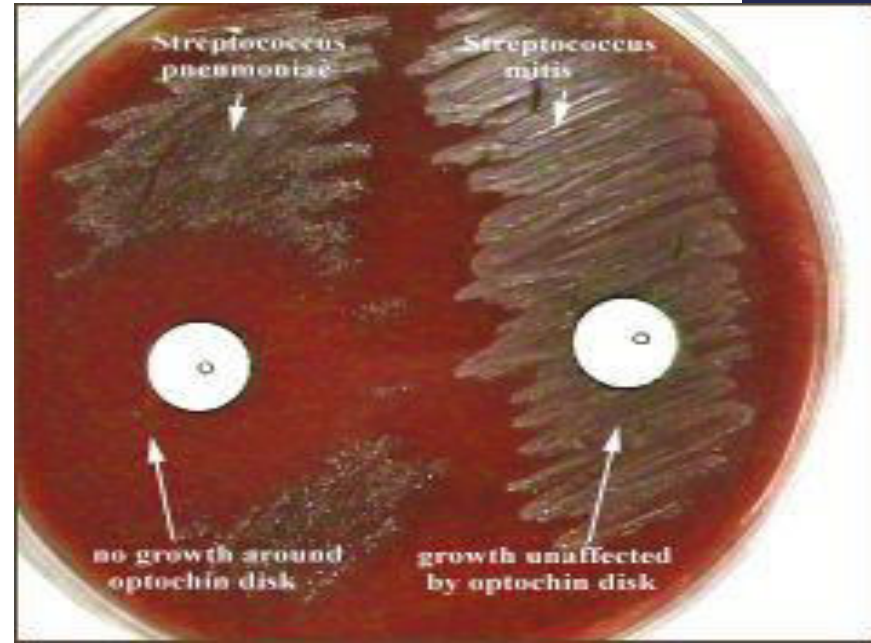
# Coagulase Test (slide method)

- Heavy suspension of organism is made on glass slide and mixed with drop of plasma
- Coagulase Positive:  
Macroscopic clumping in 10 seconds or less in coagulated plasma drop
- Coagulase Negative:  
No clumping



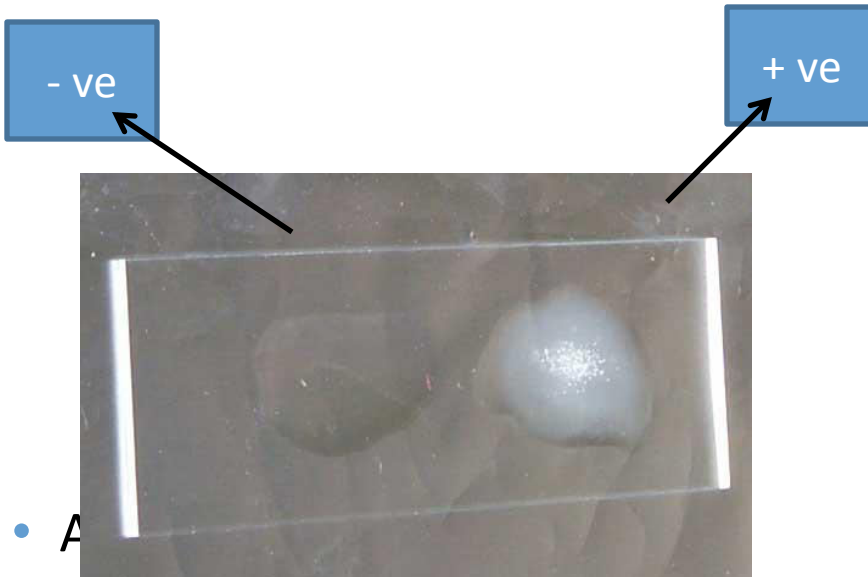
# Coagulase Negative Staphylococci

- Novobiocin Disk:
- *Staphylococcus saprophyticus* → Resistant
- *Staphylococcus epidermidis* → Sensitive



# Streptococci

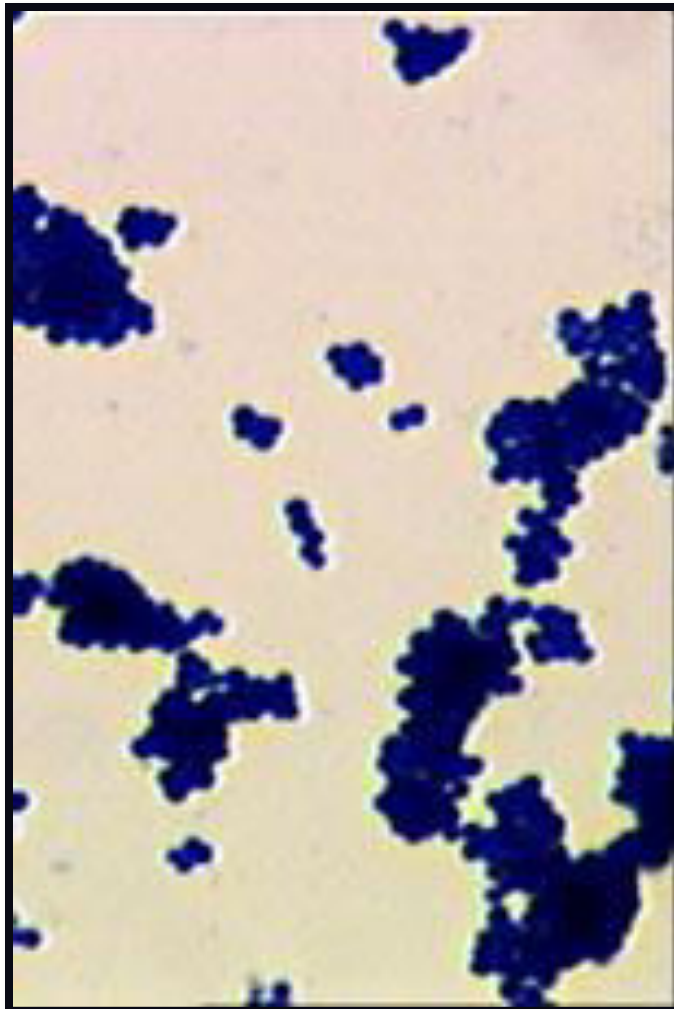
- All are Gram Positive Cocci arranged in chains (short or long)





# Gram Stain

Staphylococci



Streptococci



# Culture

- Fastidious organism → no growth on NA
- Grows well on BA
- Facultative anaerobe
- Grow at 37C need 24 to 48 hour .

# Streptococcus Classification

- Alpha hemolytic

- *S. viridans*
- *S. pneumoniae*

- Beta hemolytic:

- Group A, B, C, G & D

Lancefield grouping according to antigens in their cell wall

- Non-hemolytic:

- Enterococcus (formally known as Group D)



# Hemolytic Streptococci

- Alpha hemolysis on BA (partial hemolysis).
- Beta hemolysis on BA (complete hemolysis).
- No hemolysis on BAP



# Alpha Hemolytic Streptococci

- Give alpha hemolysis on BA.
- It Can be divided in to 2:
  - Viridans group
  - *S.pneumoniae*



**Streptococcus viridans**



***S. pneumoniae***



## Viridans Streptococci

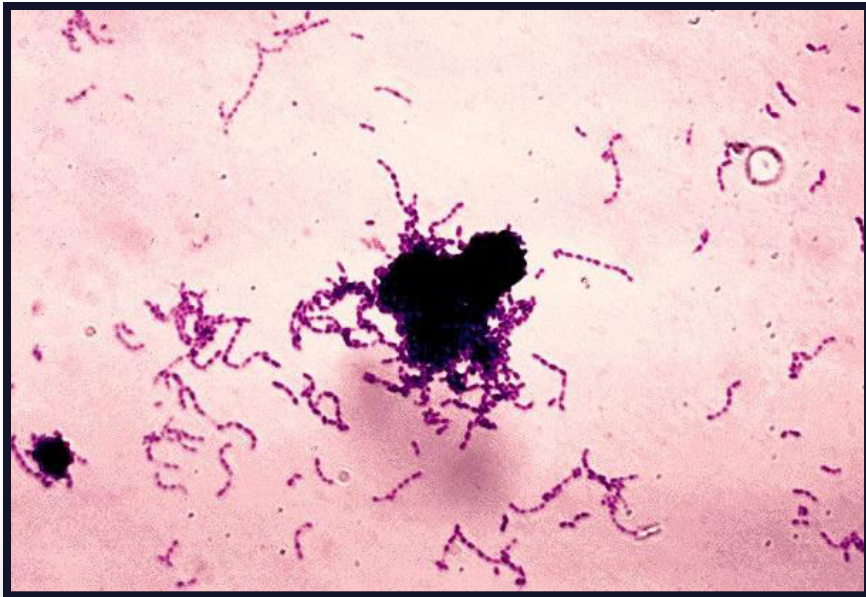
- Alpha haemolysis
- Not Capsulated
- Optochin → resistant
- Part of normal flora of upper respiratory and GI

## *S. pneumoniae*

- Alpha haemolysis
- Capsulated, Translucent or mucoid
- Optochin → sensitive
- Not part of NF

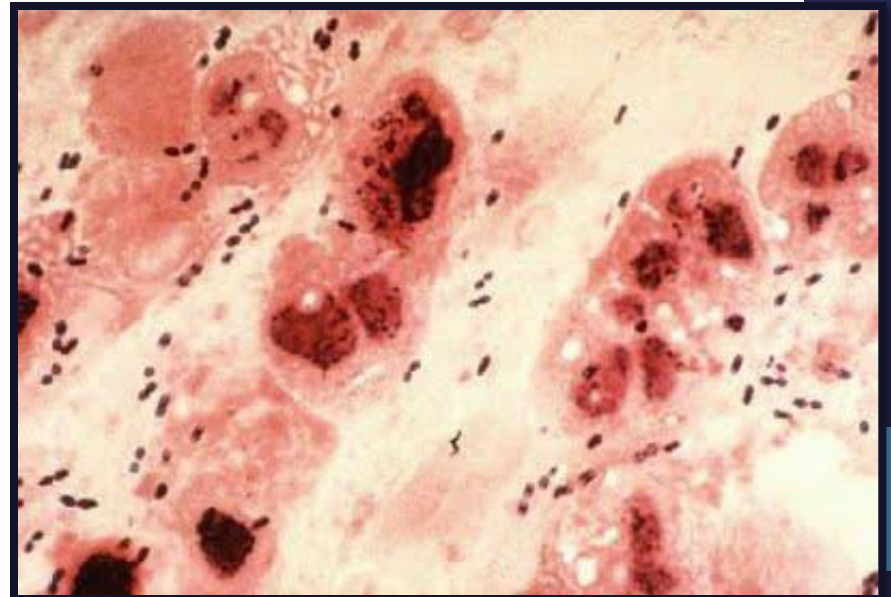
## Viridans Streptococci

- GPC in chains



## *S. pneumoniae*

- GP diplococci



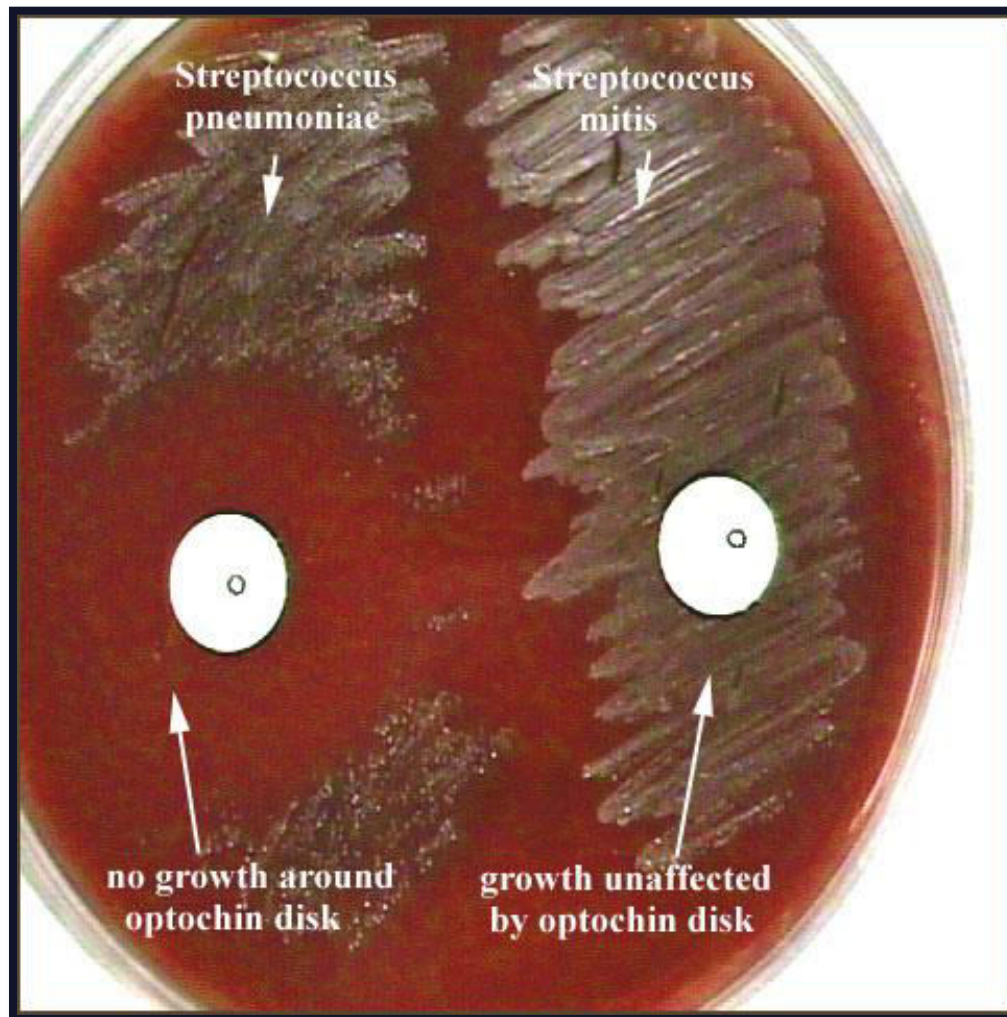
# 1. Optochin Test

- Zone of inhibition of 14 mm or more → sensitive → S. pneumoniae
- No zone of inhibition → *S. viridans*

Always don on a BA.



# Optochin Disk



# Rapid Detection of *S. pneumoniae*

- Rapid latex and co-agglutination tests are available to detect capsular pneumococcal antigen in CSF or other sterile body fluids.

