

Streptococcus

Lab. No.2



Identification of Gram's +ve Cocci

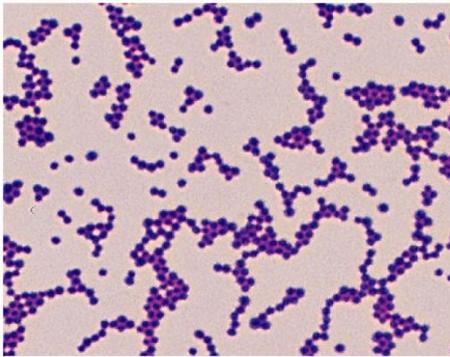
1. Microscopical Appearance:(Gram's Stain)

Gram's +ve Cocci

Irregular Clusters

Tetrads

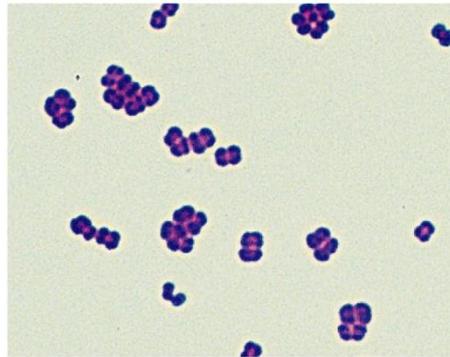
Chains or Pairs



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Staphylococci

Manal Al Khulaifi



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Micrococci



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Streptococci

■ Characters of Streptococci

- Gram positive cocci
- 1 μ m in diameter
- Chains or pairs
- Usually capsulated
- Non motile
- Non spore forming
- Facultative anaerobes
- Fastidious
- Catalase negative (Staphylococci are catalase positive)

Classification of Streptococci

- Streptococci can be classified according to:
 - Oxygen requirements
 - Anaerobic (*Peptostreptococcus*)
 - Aerobic or facultative anaerobic (*Streptococcus*)
 - Serology (Lanciefield Classification)
 - Hemolysis on Blood Agar (BA)

Identification of Sterptococci

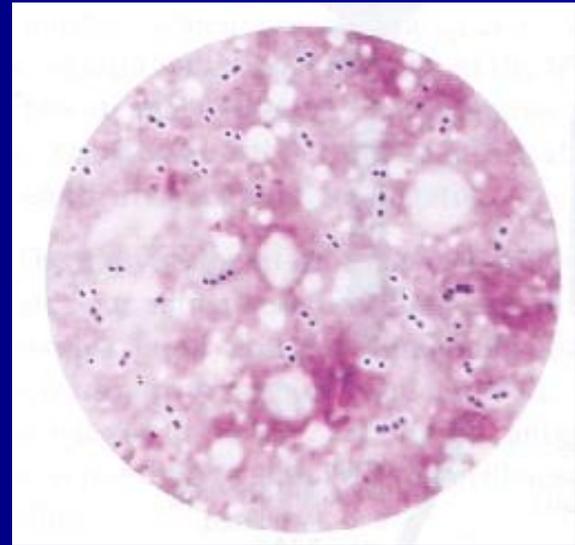
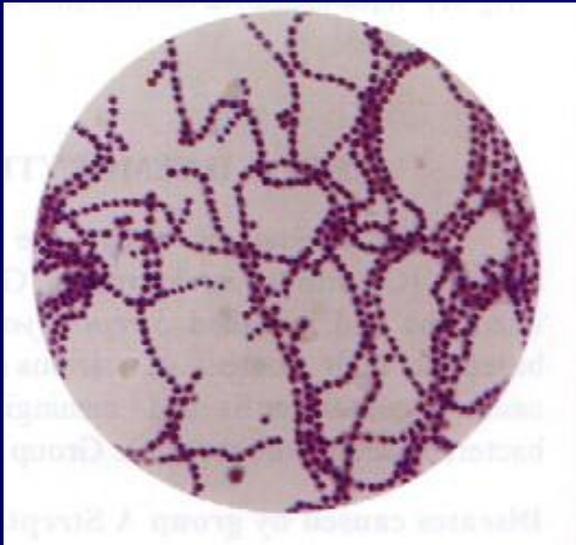
- Gram's Stain:

Gram's +ve cocci arranged in:

chains

or

pairs (*S. Pneumonia*)



- Macroscopical Examination:

Transparent pin point colonies

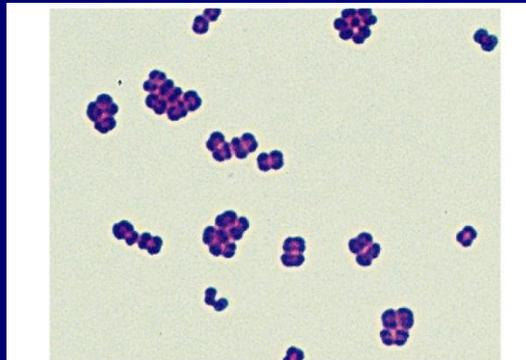
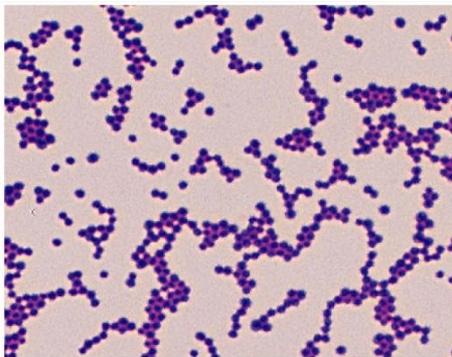
Catalase Test

Gram's +ve Cocci

Irregular Clusters

Tetrads

Chains or Pairs



Staphylococci

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Micrococci

Streptococci

Catalase +ve

Catalase -ve

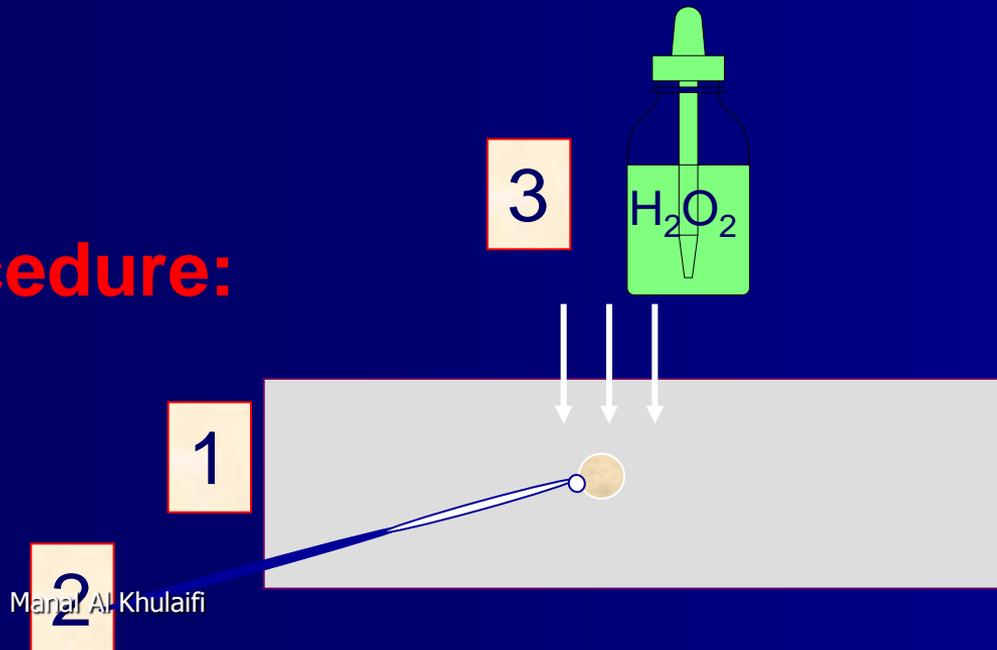
Catalase Test

- Differentiative test to separate **Staphylococci and Micrococci which are catalase +ve** from **Sterptococci which are catalase -ve.**

Principle:



Procedure:



Catalase Test

Results:

Positive test: rapid appearance of gas bubbles.



Catalase +ve



Staphylococci or Micrococci

Manal Al Khulaifi



Catalase -ve



Streptococci

Growth on Blood Agar

Sterptococci are divided into three main groups according to its action on erythrocytes:

1. β -hemolytic Sterptococci.
2. α -hemolytic Sterptococci.
3. γ -hemolytic Sterptococci.

Growth on Blood Agar

- β -hemolytic Streptococci:



- It causes **complete hemolysis** to RBCs leading to formation of clear zone around the colonies

- Example: *Strept. Pyogenes*

Manal Al Khulaifi (group A β -hemolytic Strept.)

Growth on Blood Agar

■ α -hemolytic Streptococci:



➤ It causes:

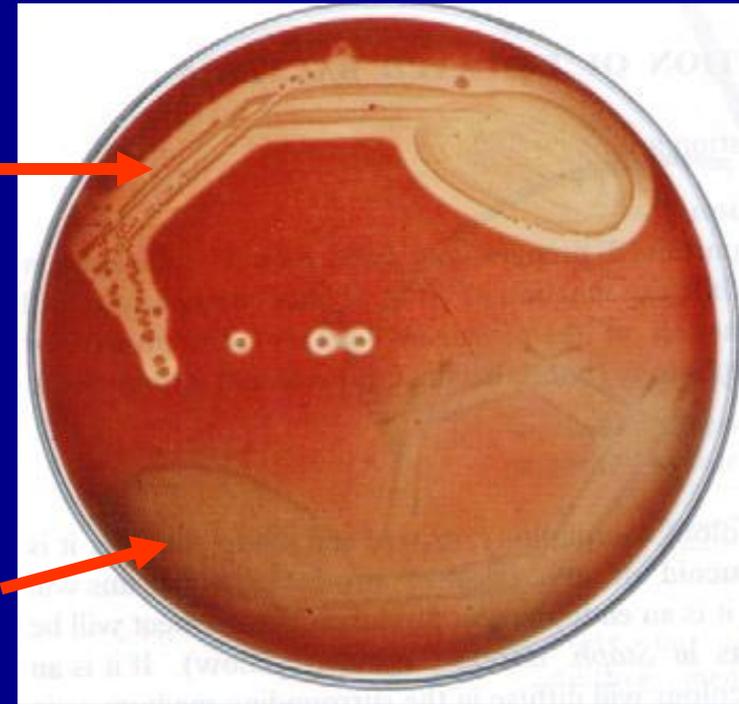
1. **Partial hemolysis** to RBCs.
2. Act enzymatically on blood pigment leading to **green discoloration** around the colonies.

➤ Example: *Strept. Pneumonia, viridans Streptococci.*

Growth on Blood Agar

β -hemolytic Sterptococci: →

α -hemolytic Sterptococci: →



Growth on Blood Agar

- γ -hemolytic Sterptococci:
 - It has **no effect** on RBCs
(Non hemolytic Sterptococci)
 - Example: *Enterococcus faecalis*



γ -hemolytic Sterptococci.

α -hemolytic Sterptococci.

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β -hemolytic Sterptococci.

Growth on Blood Agar

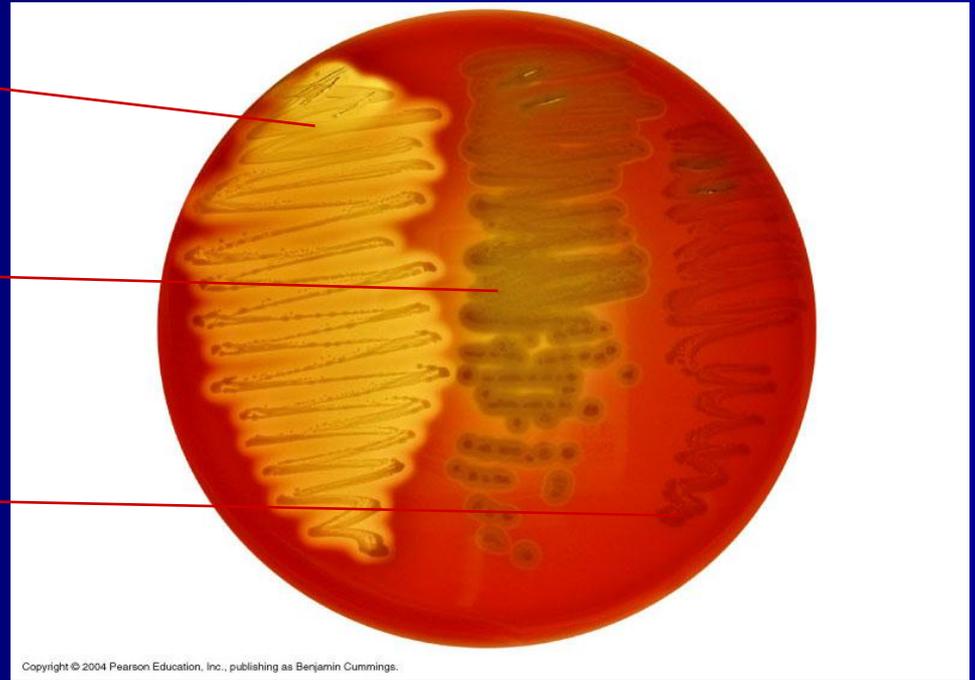


Hemolysis on Blood agar

β -hemolysis

α -hemolysis

γ -hemolysis



β -hemolytic Streptococci

Definitive test to differentiate between

S. Pyogenes & Non group A β -hemolytic Streptococci

Bacitracin Sensitivity Test:

Principle:

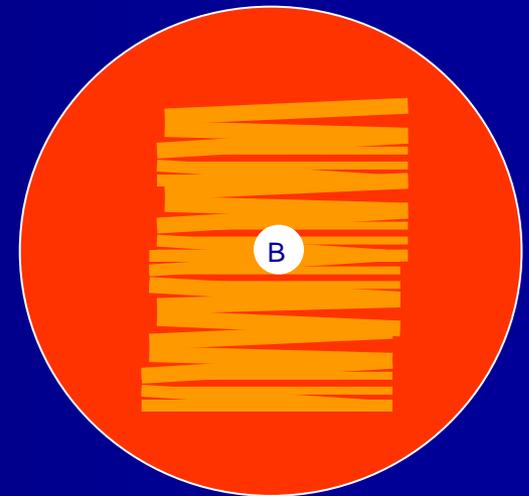
A low conc. of Bacitracin (0.04 units) will selectively inhibit the growth of *S.pyogenes* giving a zone of inhibition around the disc

β -hemolytic Streptococci

Bacitracin Sensitivity Test:

Procedure:

1. Inoculate blood agar plate with the test organism.
2. Aseptically apply Bacitracin disc onto the center of the streaked area.
3. Incubate the plate at 35°C for 18 hrs.

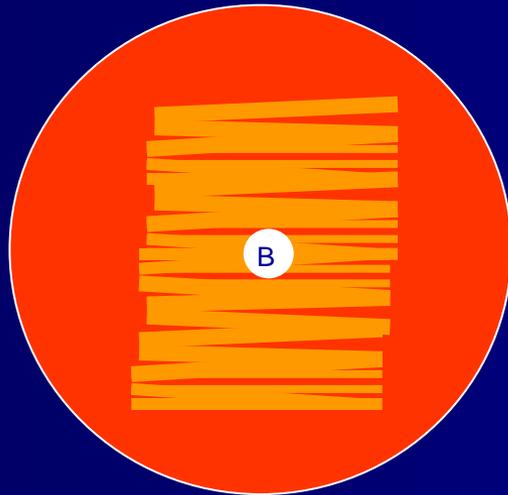


β -hemolytic Streptococci

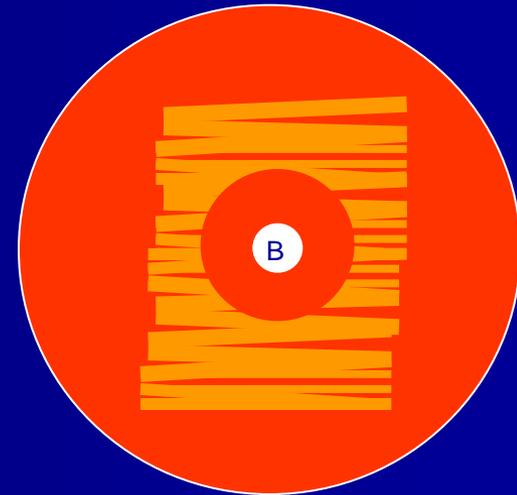
Bacitracin Sensitivity Test:

Results:

Positive test: any zone of inhibition around the disc.



Bacitracin Resistant



Bacitracin Sensitive

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Non group A β -hemolytic Streptococci

S. Pyogenes

CAMP test

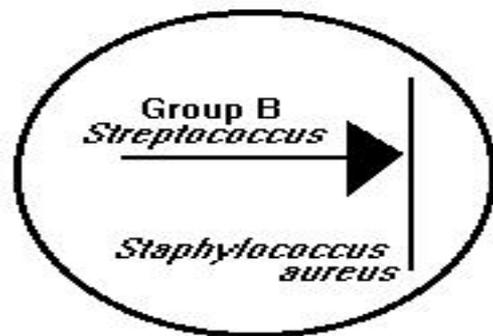
■ Principle:

- Group B streptococci produce extracellular protein (CAMP factor)
- CAMP act synergistically with staph. β -lysin to cause lysis of RBCs

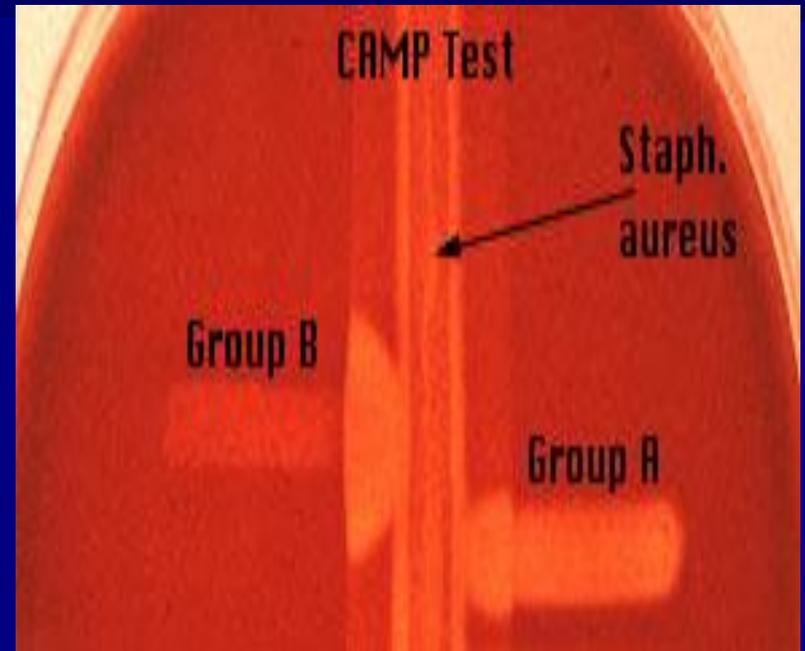
■ Procedure:

- Single streak of *Streptococcus* to be tested and a *Staph. aureus* are made perpendicular to each other
- 3-5 mm distance was left between two streaks
- After incubation, a positive result appear as an arrowhead shaped zone of complete hemolysis
- *S. agalactiae* is CAMP test positive while non gp B streptococci are negative

Positive Control:
S. agalactiae



Negative Control:
Group A or Group D Strep.



Group A streptococci

■ Pathogenesis and Virulence Factors

Structural components

M protein M

Lipoteichoic acid & F protein

Hyaluronic acid capsule, which acts to camouflage the bacteria

Enzymes

Streptokinases

Deoxynucleases

C5a peptidase

Pyrogenic toxins

Streptolysins

Streptolysin O lyse red blood cells, white blood cells, and platelets

Streptolysin S

α -hemolytic Streptococci

Definitive test to differentiate between

S.Pneumoniae & *Viridans Streptococci*

1. Optochin Sensitivity Test:

Principle:

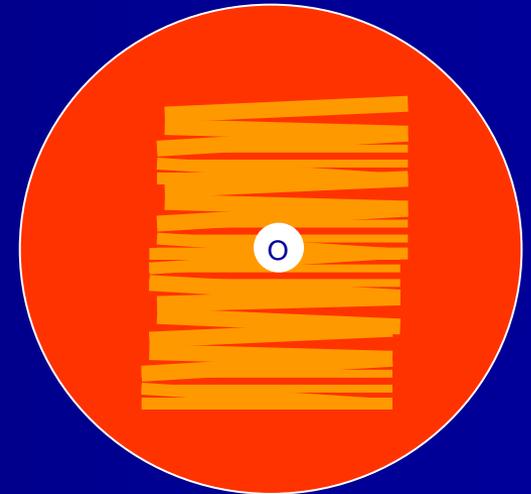
S.Pneumoniae is inhibited by less than 5 $\mu\text{g/ml}$ Optochin reagent giving a zone of inhibition more than 15 mm in diameter.

α -hemolytic Streptococci

1. Optochin Sensitivity Test:

Procedure:

1. Inoculate blood agar plate with the test organism.
2. Aseptically apply Optochin disc onto the center of the streaked area.
3. Incubate the plate at 35°C for 18 hrs.
4. Accurately measure the diameter of the inhibition zone around the disc.

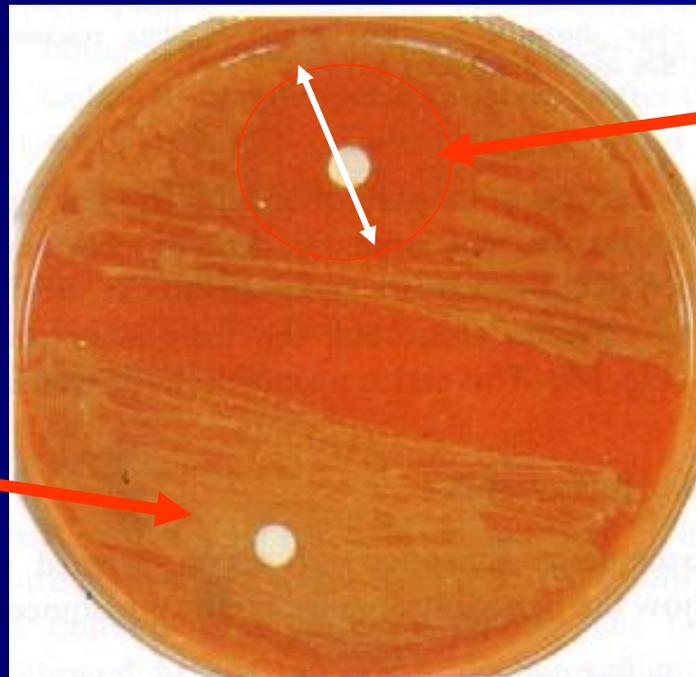


α -hemolytic Streptococci

1. Optochin Sensitivity Test:

Results:

Positive test: inhibition zone more than 15 mm in diameter.



Optochin sensitive



S.pneumoniae

Optochin resistant

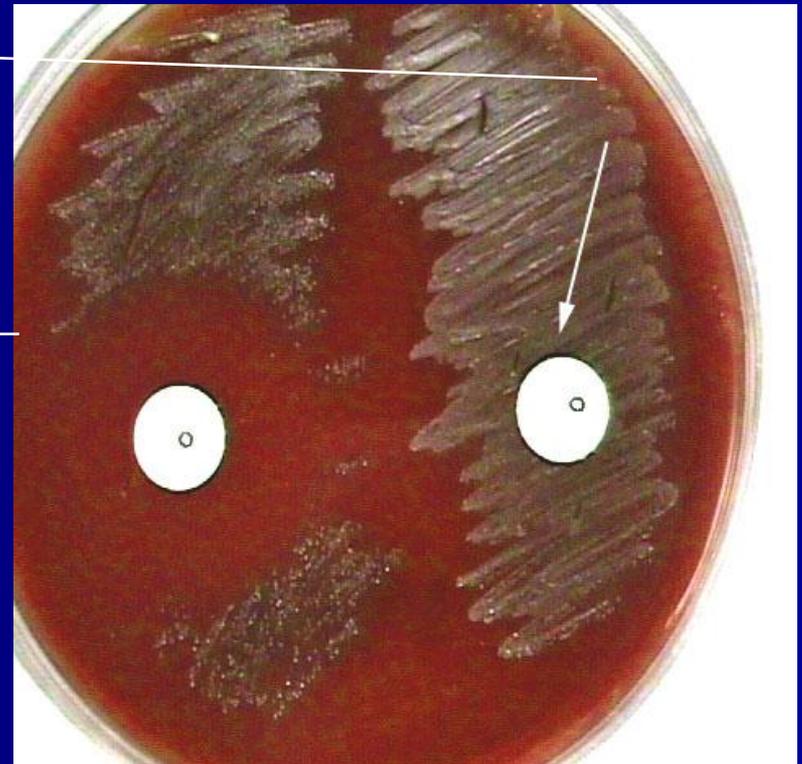


Viridans Streptococci

Optochin Susceptibility Test

Optochin resistant
S. viridans

Optochin susceptible
S. pneumoniae

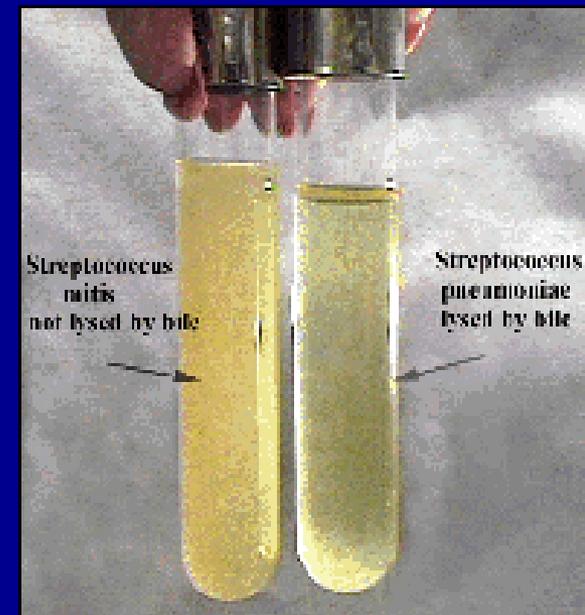


α -hemolytic Streptococci

2. Bile Solubility Test:

Principle:

S. Pneumoniae produce a self-lysing enzyme to depress the growth of old colonies. The presence of bile salt accelerate this process.



α -hemolytic Streptococci

2. Bile Solubility Test:

Procedure:

2

Add 1 ml 10% bile salt solution



1

10 ml broth culture of the test organism

3. Incubate at 37°C for 15 min .

4. Observe for the visible clearing of the turbid culture.

α -hemolytic Streptococci

2. Bile Solubility Test:

Results:

Positive test: Visible clearance of the turbid culture.

Visible clearance



S.Pneumoniae



Remain turbid



Viridans Streptococci

Differentiation between β -hemolytic streptococci

| | Hemolysis | Bacitracin sensitivity | CAMP test |
|----------------------|-----------|------------------------|-----------|
| <i>S. pyogenes</i> | β | Susceptible | Negative |
| <i>S. agalactiae</i> | β | Resistant | Positive |

Differentiation between α -hemolytic streptococci

| | Hemolysis | Optochin sensitivity | Bile solubility | Inulin Fermentation |
|--|-----------|---------------------------|-----------------|---------------------|
| <i>S. pneumoniae</i> | α | Sensitive (≥ 14 mm) | Soluble | Not ferment |
| <i>Viridans strep</i> Manal Al Khulaifi | α | Resistant (≤ 13 mm) | Insoluble | Ferment |

γ -hemolytic Streptococci

Definitive test for *Enterococcus faecalis*

Growth on MacConkey's agar:

Principle:

- ❖ MacConkey's agar is a selective medium for Gram's -ve bacteria.
- ❖ It contains bile salt and crystal violet to inhibit the growth of Gram's +ve bacteria.
- ❖ *Enterococcus faecalis* is the only Streptococcus species which can grow on MacConkey's agar giving pink colonies.

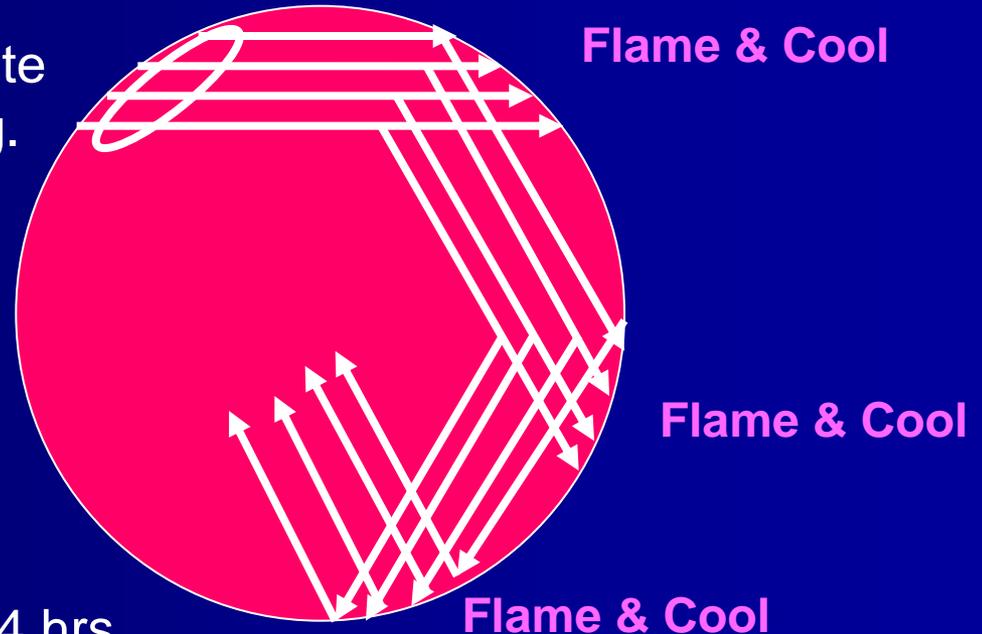
γ -hemolytic Streptococci

Definitive test for *Enterococcus faecalis*

Growth on MacConkey's agar:

Procedure:

1. Inoculate MacConkey's agar plate with the test organism by streaking.



2. Incubate the plate at 35°C for 24 hrs.

γ -hemolytic Streptococci

Definitive test for *Enterococcus faecalis*

Growth on MacConkey's agar:

Results:



Growth of pink colonies



Enterococcus faecalis

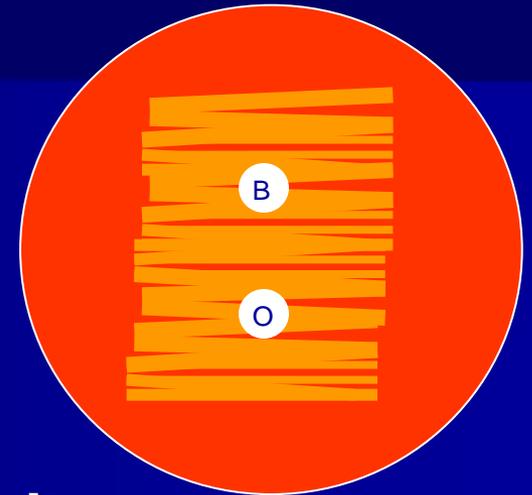
No Growth



Other Strain of Streptococci

Practical Work

1. Gram's Stain (spots)
2. Catalase test
3. Blood agar plate.
4. Bacitracin & Optochin Sensitivity.
5. MacConkey's agar plate.



Identification of Sterptococci

| | β -hemolytic Sterptococci | α -hemolytic Sterptococci | γ -hemolytic Sterptococci |
|-------------------------|-------------------------------------|--|-------------------------------------|
| Gram's Stain | Gram's +ve Cocci arranged in chains | | |
| Catalase test | - ve | - ve | - ve |
| Growth on blood agar | Complete hemolysis | 1. Partial hemolysis 2. Green pigmentation | Non hemolytic |

Identification of Sterptococci

| | β -hemolytic Sterptococci | α -hemolytic Sterptococci | γ -hemolytic Sterptococci |
|-------------------------------|---|----------------------------------|----------------------------------|
| Bacitracin sensitivity | <pre> graph TD A[Inhibition zone] --> B[Bacitracin sensitive] B --> C["S.pyogenes"] D[No zone] --> E[Bacitracin resistant] E --> F["Non group A beta-hemolytic Strept."] </pre> | - | - |

Identification of Sterptococci

| | β -hemolytic Sterptococci | α -hemolytic Sterptococci | | γ -hemolytic Sterptococci |
|-----------------------------|---------------------------------|--|--|----------------------------------|
| Optochin sensitivity | - | <p style="text-align: center;"> </p> <p style="text-align: center;"> </p> <p style="text-align: center;"> </p> | - | |
| Bile Solubility | - | <p style="text-align: center;"> </p> <p style="text-align: center;"><i>S.pneumoniae</i></p> | <p style="text-align: center;"> </p> <p style="text-align: center;"><i>Viridans Streptococci</i></p> | - |

Identification of Sterptococci

| | β -hemolytic Sterptococci | α -hemolytic Sterptococci | γ -hemolytic Sterptococci |
|----------------------------|---------------------------------|----------------------------------|--|
| Growth on MacConkey's Agar | No Growth | No Growth | Growth of pink colonies ↓ <i>Enterococcus faecalis</i> |

Outline of differentiation between Gram-Positive cocci

