

## Practical 3

### II- Streptococci

#### Objective:

1. Use of blood agar to differentiate between  $\alpha$ ,  $\beta$ , and  $\gamma$  hemolytic streptococci.
2. To know Gram reaction, shape and arrangement of streptococci.
3. To differentiate between group A and non group A streptococci using bacitracin sensitivity
4. To identify *Strep. agalactiae* by CAMP test.
5. To differentiate between *Streptococcus pneumoniae* and viridans streptococci using optochin sensitivity and bile solubility.

#### Required materials:

1. Overnight cultures of *Strep. pyogenes*, *Strep. agalactiae*, *Strep. pneumoniae*, *Strep. viridans* and *S. aureus*.
2. Gram stain "dyes and reagents", filter paper, slides, immersion oil
3. 20 plates of blood agar
4. Bacitracin disks
5. Optochin disks
6. tubes containing 5 ml of 10% bile salts

#### Characters of Streptococci

Gram positive cocci  
1  $\mu$ 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 (Lancefield Classification)

Divides the streptococci into serotype groups based on the bacteria's antigens (A-H, K-V) (C- carbohydrate antigen of cell wall)

- Lancefield groups A and B include the significant streptococcal pathogens of humans

Beta hemolytic streptococci

There is Non-groupable streptococci ; *S. pneumoniae* (pneumonia)  
and viridans streptococci

e.g. *S. mutans* that Causing dental carries

### Hemolysis on Blood Agar (BA)

#### $\alpha$ -hemolysis

Partial hemolysis

Green discoloration around the colonies

e.g. non-groupable streptococci (*S. pneumoniae* & *S. viridans*)

#### $\beta$ -hemolysis

Complete hemolysis

Clear zone of hemolysis around the colonies

e.g. Group A & B (*S. pyogenes* & *S. agalactiae*)

#### $\gamma$ -hemolysis

No lysis

e.g. Group D (*Enterococcus spp*)

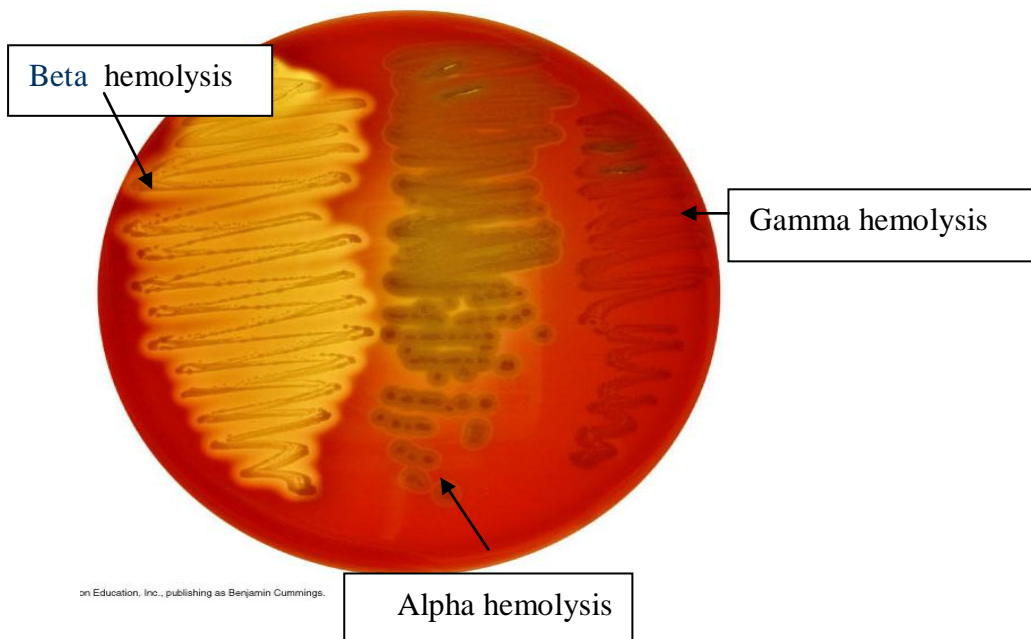




TABLE 13-1 Medically Important Streptococci

Type species	Lancefield serogroup	Normal habitat	Significant human disease
<i>S pyogenes</i>	A	Humans,	Acute pharyngitis and others
<i>S agalactiae</i>	B	Cattle, humans	Neonatal meningitis and sepsis and infections in adults
<i>S equisimilis</i>	C	Wide human and animal distribution	Endocarditis, bacteremia, pneumonia, meningitis, mild upper respiratory infection
<i>E faecalis</i> <i>S bovis (nonenterococcus)</i>	D	Human and animal intestinal tracts, dairy products bacteremia	Biliary or urinary tract infection, endocarditis,
<i>S anginosus</i>	F, G <sup>a</sup>	Humans, animals	Subcutaneous or organ abscesses, endocarditis, mild upper respiratory infection
<i>S sanguis</i> <sup>b</sup>	H	Humans	Endocarditis, caries
<i>S salivarius</i>	K	Humans	Endocarditis, caries
None	O	Humans	Endocarditis
<i>S suis</i>	R	Swine	Meningitis
"viridans" <i>S mitis</i> , <i>S mutans</i> <sup>c</sup>	None identified	Humans	Caries, endocarditis
Anaerobic or micro-aerophilic	None identified	Wide human and animal distribution	Brain and pulmonary abscesses, gynecologic infections
<i>S pneumoniae</i>	None identified	Humans	Lobar pneumonia and others

<sup>a</sup> Strains of the "S milleri" group (*S constellatus*, *S intermedius*, *S anginosus*, *minute strains*) may possess antigens of groups A, C, F, or G, or no identifiable Lancefield group antigens; a heterogeneous group, genetically related but with a wide variety of phenotypic and biochemical characteristics

<sup>b</sup> Disparate grouping undergoing further definition.

<sup>c</sup> Other viridans streptococci (*S sanguis*, *S salivarius* "S milleri," *S bovis*) have identified group antigen(s); nutritionally variant streptococci may be included in this diverse category.



### **Group A streptococci**

Include only *S. pyogenes*

### **Pathogenesis and Virulence Factors**

#### **Structural components**

*M protein M*, which interferes with opsonization and lysis of the bacteria

*Lipoteichoic acid & F protein* → adhesion

*Hyaluronic acid capsule*, which acts to camouflage the bacteria

#### **Enzymes**

*Streptokinases*

*Deoxynucleases*

*C5a peptidase*

*Pyrogenic toxins* that stimulate macrophages and helper T cells to release cytokines

*Streptolysins*

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

*Streptolysin S*

### **Some Disease caused by *S. pyogenes***

*Pharyngitis* (“strep throat”)-inflammation of the pharynx

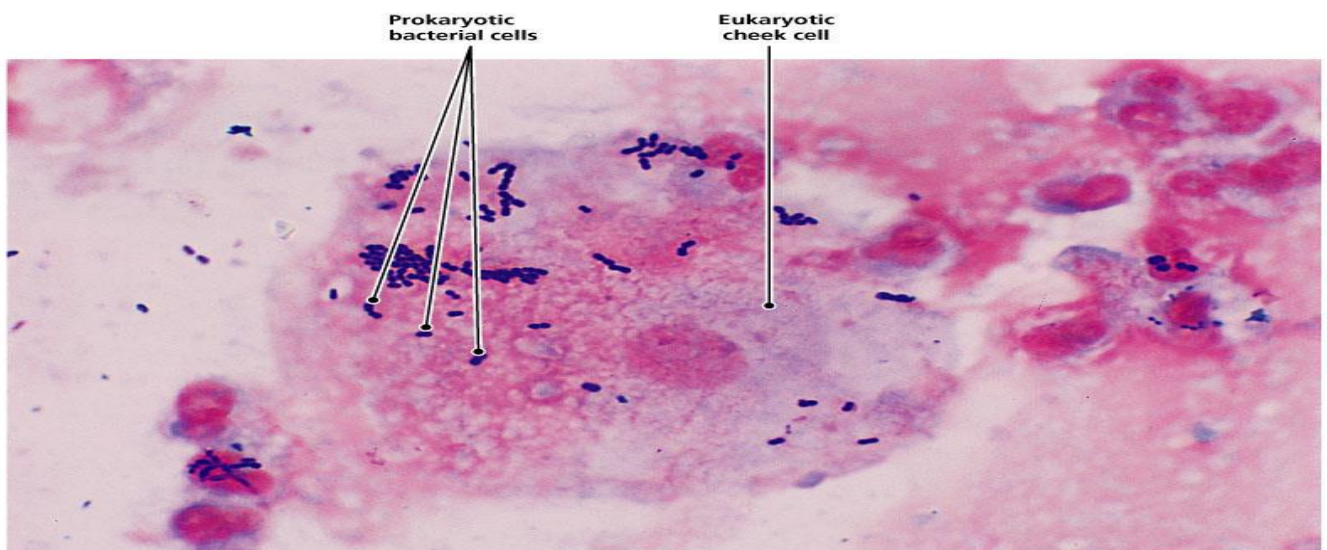
*Skin infection, Impetigo*

*Scarlet fever-rash* that begins on the chest and spreads across the body

### **Experimental:**

- 1- Gram stain of streptococci
- 2- Hemolysis on blood agar ( $\alpha$ ,  $\beta$ , and  $\gamma$  hemolysis)
- 3- Bacitracin susceptibility test
- 4- CAMP test
- 5- Optochin susceptibility test
- 6- Bile solubility test

### **Microscopial examination**

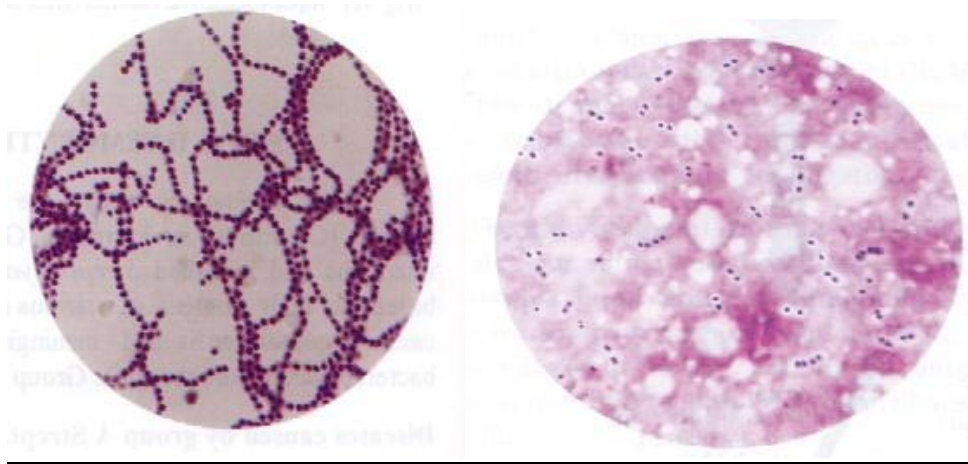




## Gram s +ve cocci arranged in:

Chains or

pairs (*S. Pneumonia*)

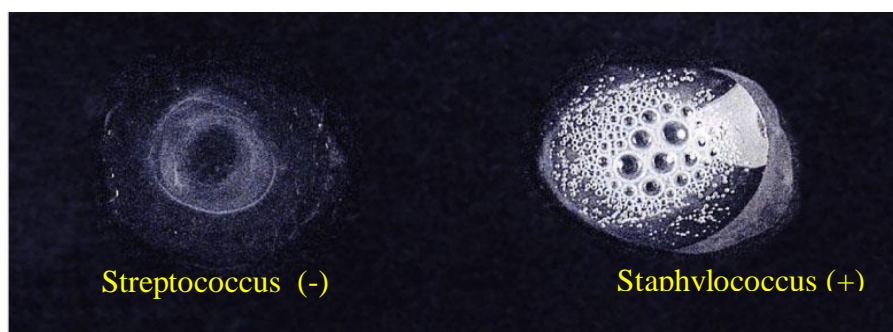
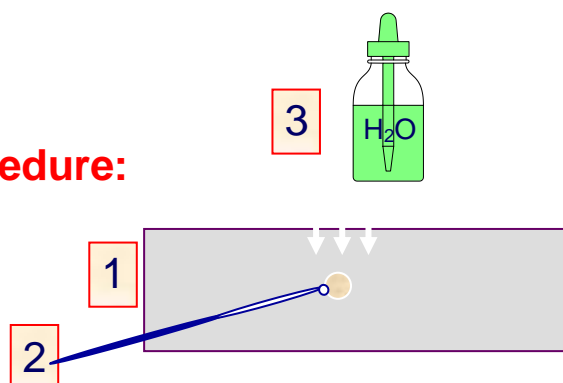


## Catalase Test

Differentiative test to separate **Staphylococci** and **Micrococci** which are catalase +ve from **Streptococci** which are catalase –ve.

**Principle:**

**Procedure:**





### **Growth on Blood Agar**

Sterptococci are divided into three main groups accorging to its action on erythrocytes:  $\beta$ -hemolytic Sterptococci\  
 $\alpha$ -hemolytic Sterptococci.  
 $\gamma$ -hemolytic Sterptococci.

#### $\beta$ -hemolytic Sterptococci

Definitive test to differentiate between  
*S.Pyogenes* & Non group A  $\beta$ -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

#### **Procedure:**

Inoculate blood agar plate with the test organism.

. Aseptically apply Bacitracin disc onto the center of the streaked area

Incubate the plate at 35oC for 18 hrs

#### **Results:**

Positive test: any zone of inhibition around the disc.



Bacitracin Resistant

Non group A  $\beta$ -hemolytic Streptococci



Bacitracin Sensitive

*S.Pyogenes*



## CAMP test

Principle:

Group B streptococci produce extracellular protein (CAMP factor)

CAMP act synergistically with staph.  $\beta$ -lysin to cause lysis of RBCs

### **Procedure:**

Single streak of *Streptococcus* to be tested and a *Staph. aureus* are made

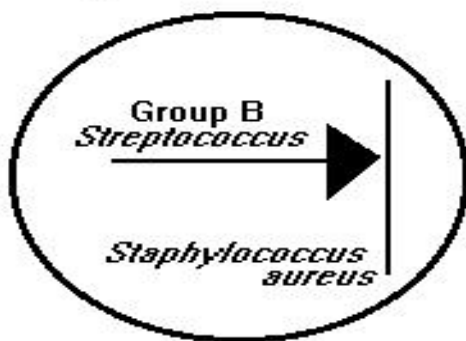
perpendicular to each other

3-5 mm distance was left between two streaks

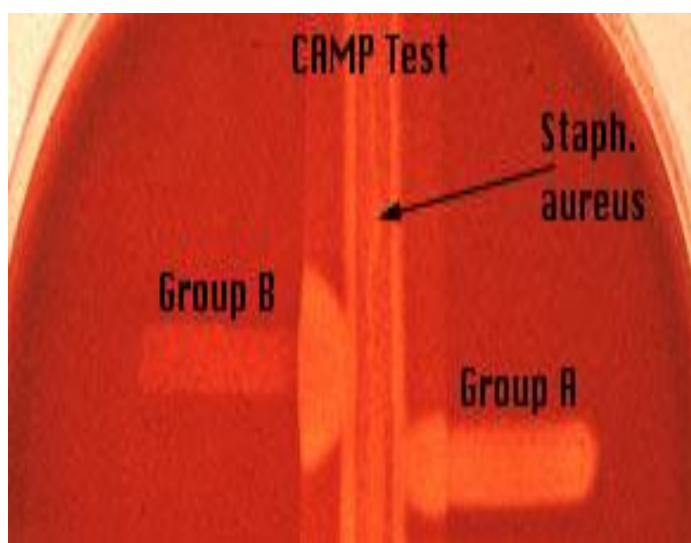
After incubation, a positive result appear as an arrowhead shaped zone of complete hemolysis

*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.**





$\alpha$ -hemolytic Streptococci  
Definitive test to differentiate between  
*S. Pneumoniae* & *Viridans Streptococci*

**Optochin Sensitivity Test:**

**Principle**

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

**Procedure**

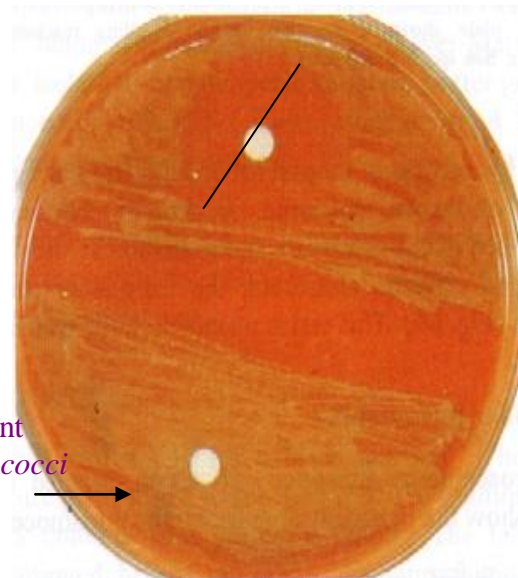
Inoculate blood agar plate with the test organism.

Aseptically apply Optochin disc onto the center of the streaked area.

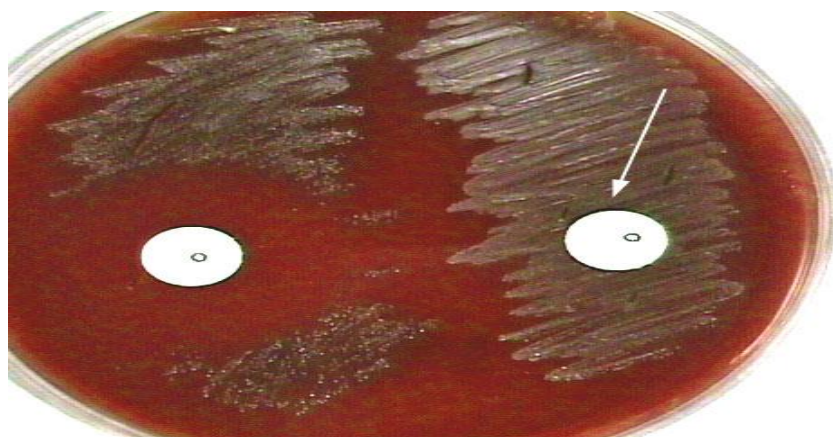
Incubate the plate at 35°C for 18 hrs.

Accurately measure the diameter of the inhibition zone around the disc.

Optochin sensitive  
*S. pneumoniae*



Optochin resistant  
*Viridans Streptococci*





## **Bile Solubility test**

### **Principle:**

*S. pneumoniae* produce a self-lysing enzyme to inhibit the growth  
The presence of bile salt accelerate this process

### **Procedure:**

Add ten parts (10 ml) of the broth culture of the organism to be tested to  
one part (1 ml) of 2% Na deoxycholate (bile) into the test tube

Negative control is made by adding saline instead of bile to the culture

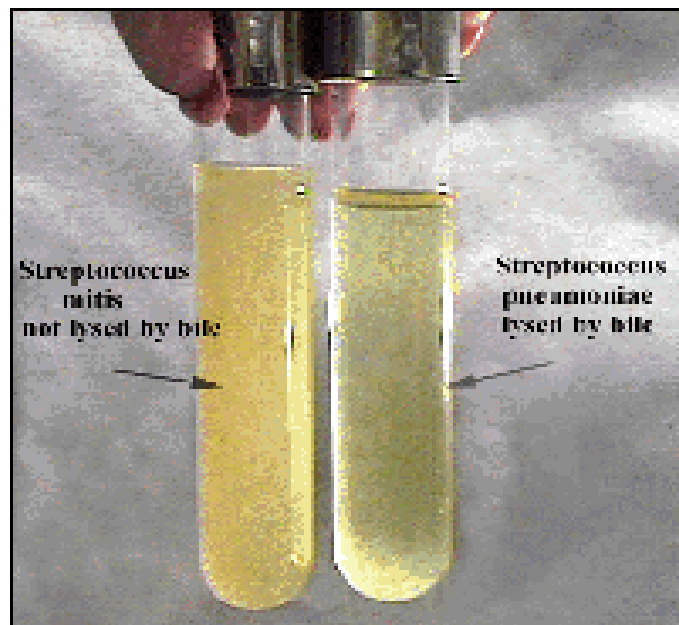
Incubate at 37°C for 15 min

Record the result after 15 min

### **Results:**

Positive test appears as clearing in the presence of bile while negative test appears as turbid

*S. pneumoniae* soluble in bile whereas *S. viridans* insoluble





## **$\gamma$ -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.

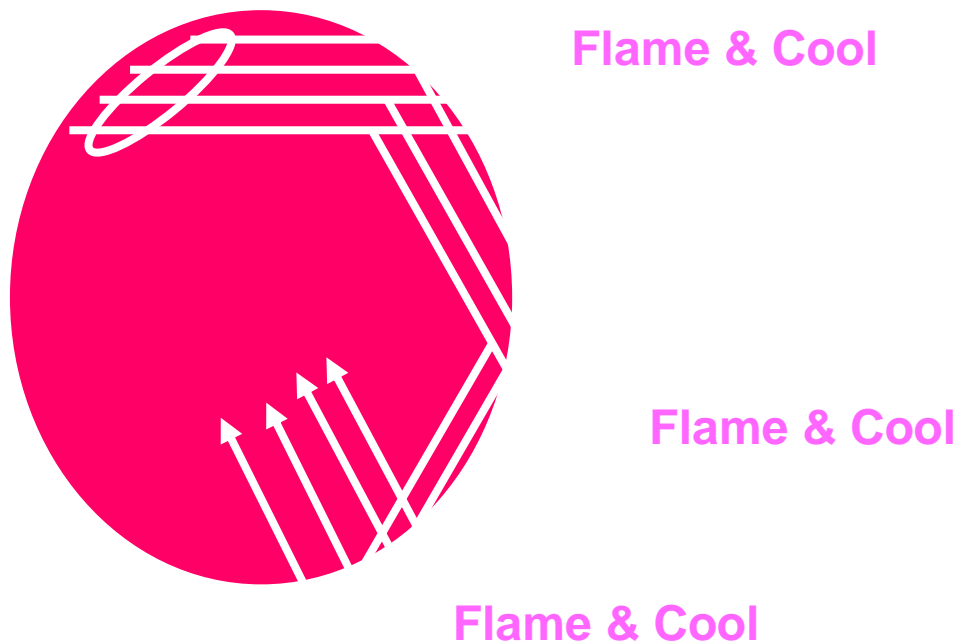
It contains bile salt and crystal violet to inhibit the growth of Gram's +ve bacteria.

*Enterococcus faecalis* is the only Streptococcus species which can grow on MacConkey's agar giving pink colonies.

#### **Procedure:**

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

Incubate the plate at 35°C for 24 hrs.



#### **Results:**

Growth of pink colonies  $\longrightarrow$  *Enterococcus faecalis*

No Growth  $\longrightarrow$  Other Strain of Streptococcus







# Gram Positive Cocci Flow Chart

