

Practical Medical Bacteriology

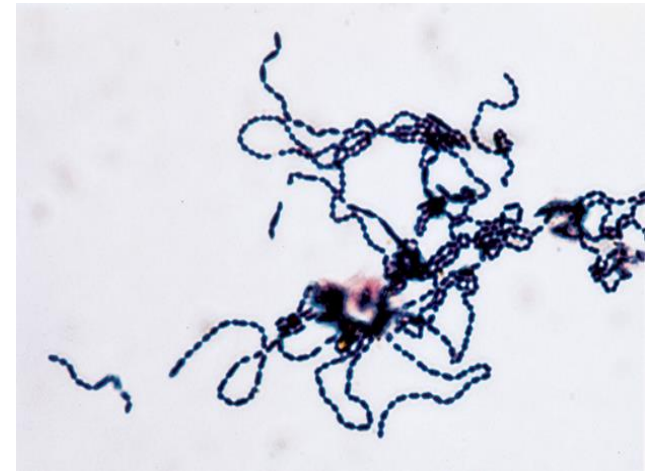
Lab 4 Laboratory Diagnosis of Streptococcal Group



2021/2022

General Characteristics of Streptococci

- Gram-positive spherical bacteria arranged in long chains; or in pairs.
- The colonies are small, ranging from pinpoint size to 2 mm in diameter,
- Some are members of the normal human microbiota ; others are associated with important human diseases
- Non-motile and do not form spores
- Some members form capsules
- Anaerobic or Facultative anaerobes
- Catalase negative
- Sensitive to drying, heat, and disinfectants



Classification of Streptococci

Streptococci can be classified according to:

- **Colony morphology** and hemolytic reactions on blood agar.
- **Serologic specificity** of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens.
- **Biochemical reactions** and resistance to physical and chemical factors.
- **Ecologic features** and Molecular genetics



Lancefield Classification of Streptococci

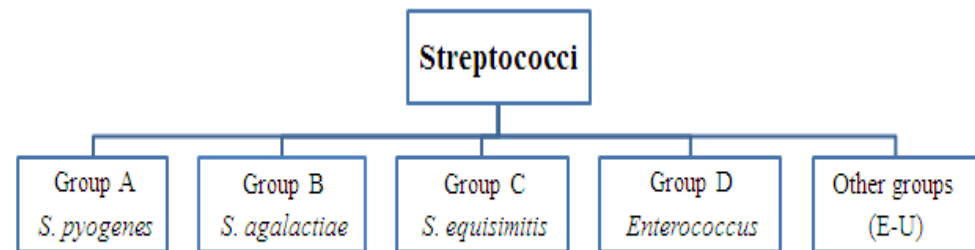
- Classification based on C- carbohydrate that contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups **A- H and K-U**.
- Typing is generally done only for groups A, B, C, F, and G which cause disease in humans.
- **Based on C- carbohydrate antigen of cell wall streptococci can be classified into two groups**

- ✓ **Groupable streptococci**

- A, B and D (more frequent)
- C, G and F (Less frequent)

- ✓ **Non-groupable streptococci**

- *S. pneumoniae* (pneumonia)
- viridans streptococci
- e.g. *S. mutans* that cause dental caries



Classification of Streptococci based on hemolysis on blood agar

➤ Hemolysis on Blood Agar

1. α -hemolysis

- Partial hemolysis
- Green discoloration around the colonies
- e.g. non- groupable Streptococci (*S.pneumoniae* & *S.viridans*)

2. β -hemolysis

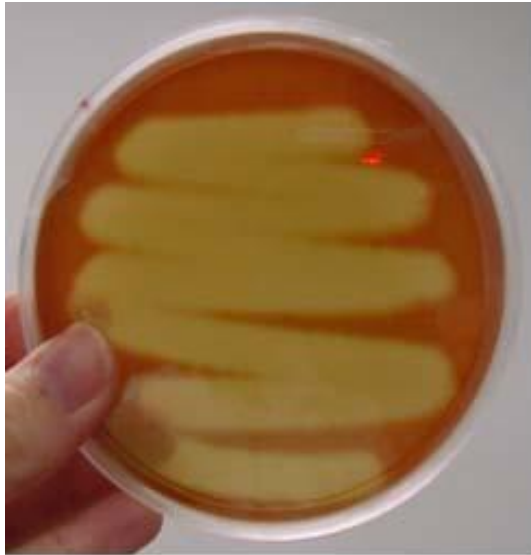
- Complete hemolysis
- Clear zone of hemolysis around the colonies
- e.g. Group A & B (*S. pyogenes* & *S. agalactiae*)

3. γ -hemolysis

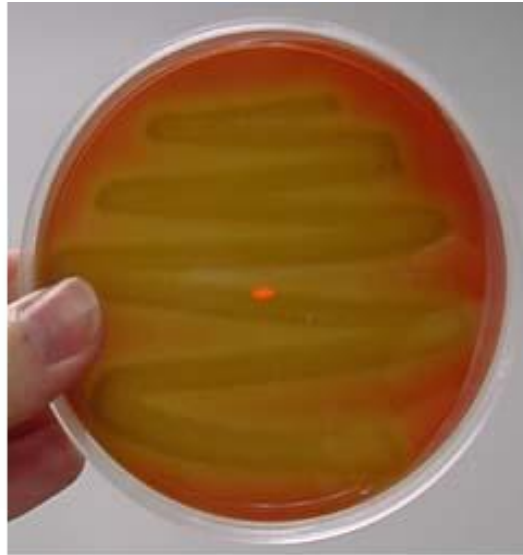
- No lysis
- e.g. Group D (*Enterococcus* spp)



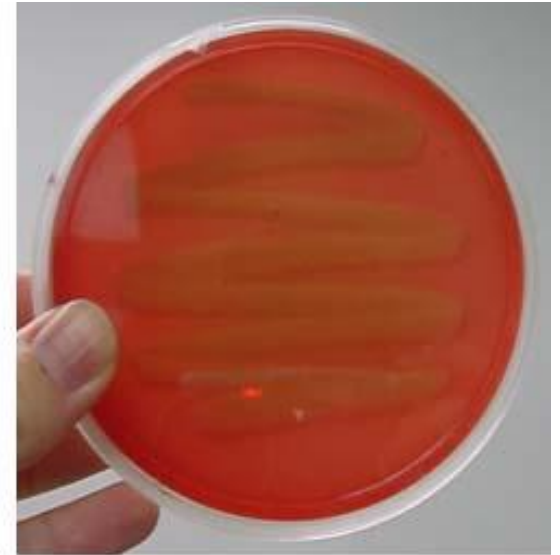
Classification of Streptococci based on hemolysis on blood agar



Beta Hemolysis



Alpha Hemolysis



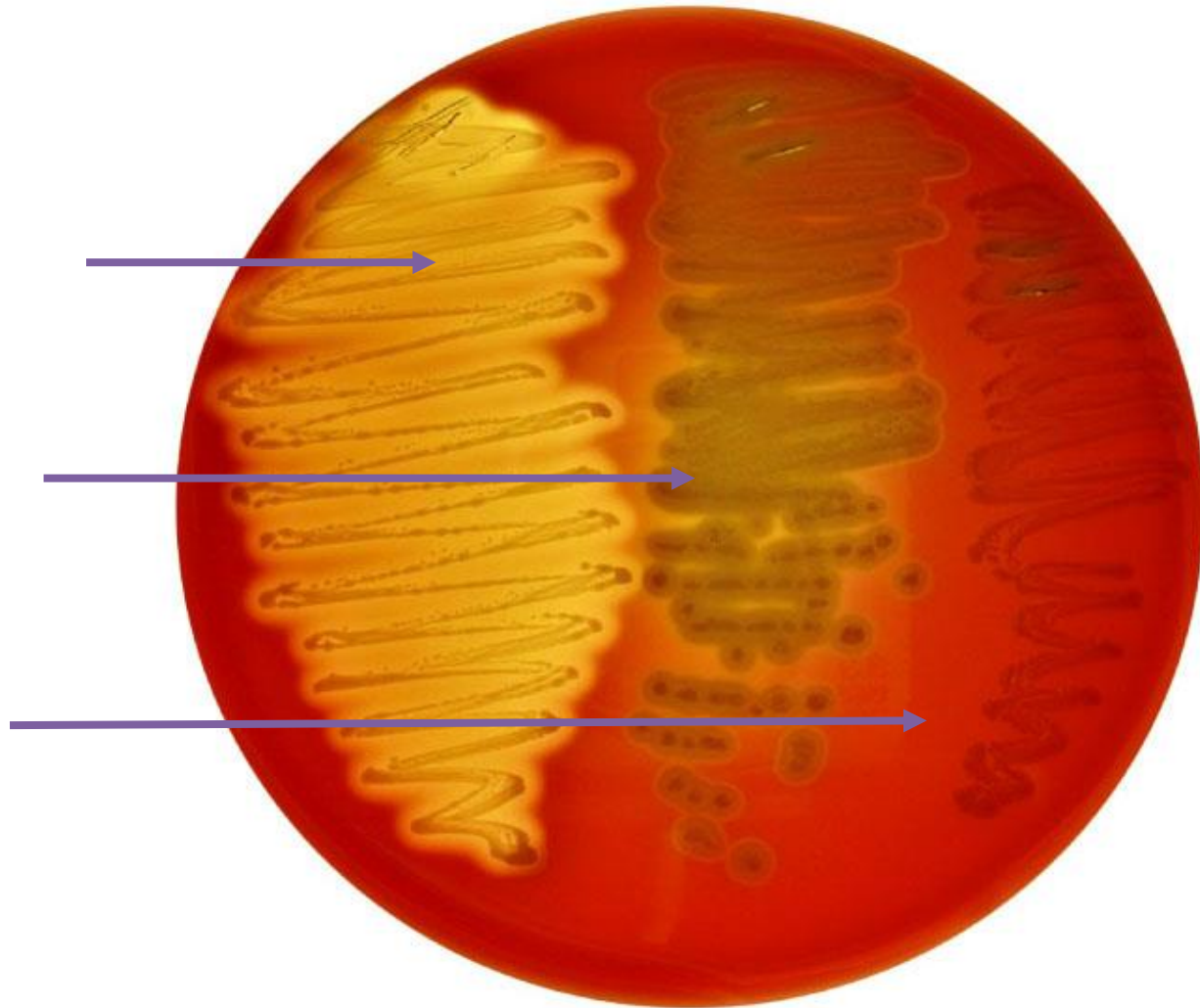
Gamma Hemolysis

Classification of Streptococci based on hemolysis on blood agar

β -hemolysis

α -hemolysis

γ -hemolysis



Laboratory diagnosis

1. Specimen:

- Throat swab, Pus swab, CSF, Blood, Urine

2. Culture:

- Blood agar
- Chocolate agar
- Other selective media

3. Biochemical tests

4. Serological methods for identification of some strains or species

5. Antibiotic sensitivity tests

6. Molecular biology methods for research



Laboratory diagnosis

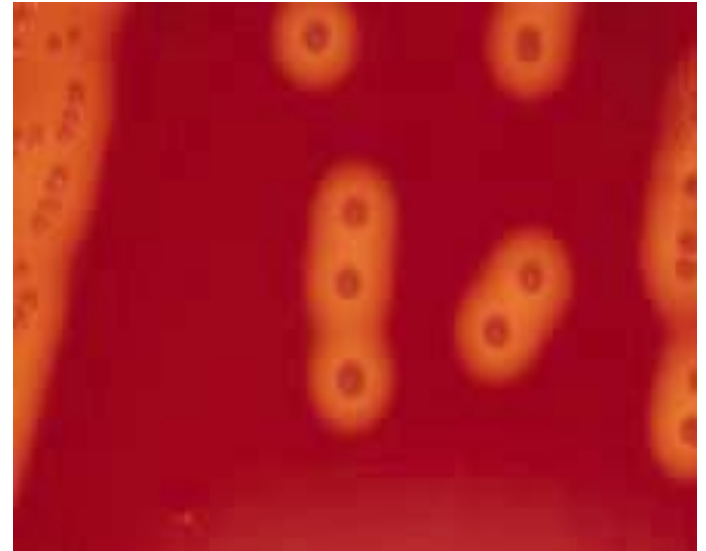
Group A β - hemolysis Streptococcus

➤ Colony morphology

- Transparent, smooth, and well-defined zone of beta- hemolysis

➤ Identification

- Catalase-negative
 - **Bacitracin-susceptible**
 - Bile-esculin–negative
 - 6.5% NaCl-negative
-
- Group A streptococci (*S. pyogenes*) is susceptible to Bacitracin disk (left); The right shows resistance



Laboratory diagnosis

Group B β - hemolysis Streptococcus

➤ Colony morphology

- Grayish-white, mucoid, creamy, narrow zone of β -hemolysis

➤ Presumptive Identification tests

- Catalase-negative

➤ **Bacitracin-resistant**

➤ Identification tests

- Bile esculin hydrolysis–negative
- Does not grow in 6.5% NaCl
- CAMP test–positive

- *S. agalactiae* shows the arrow-shaped hemolysis near the staphylococcus streak, showing a positive test for CAMP factor

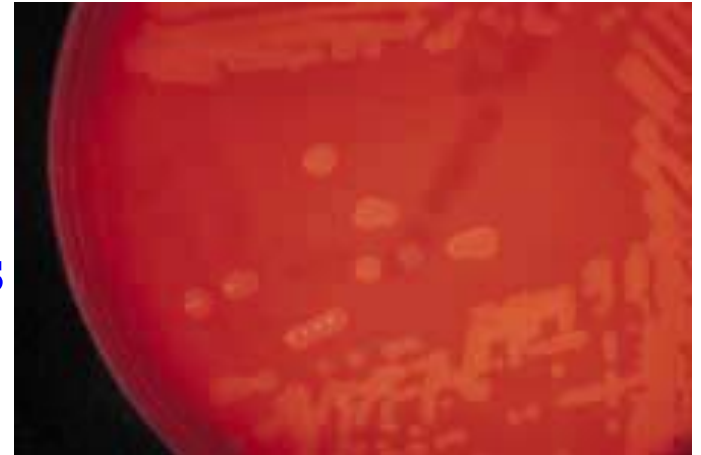
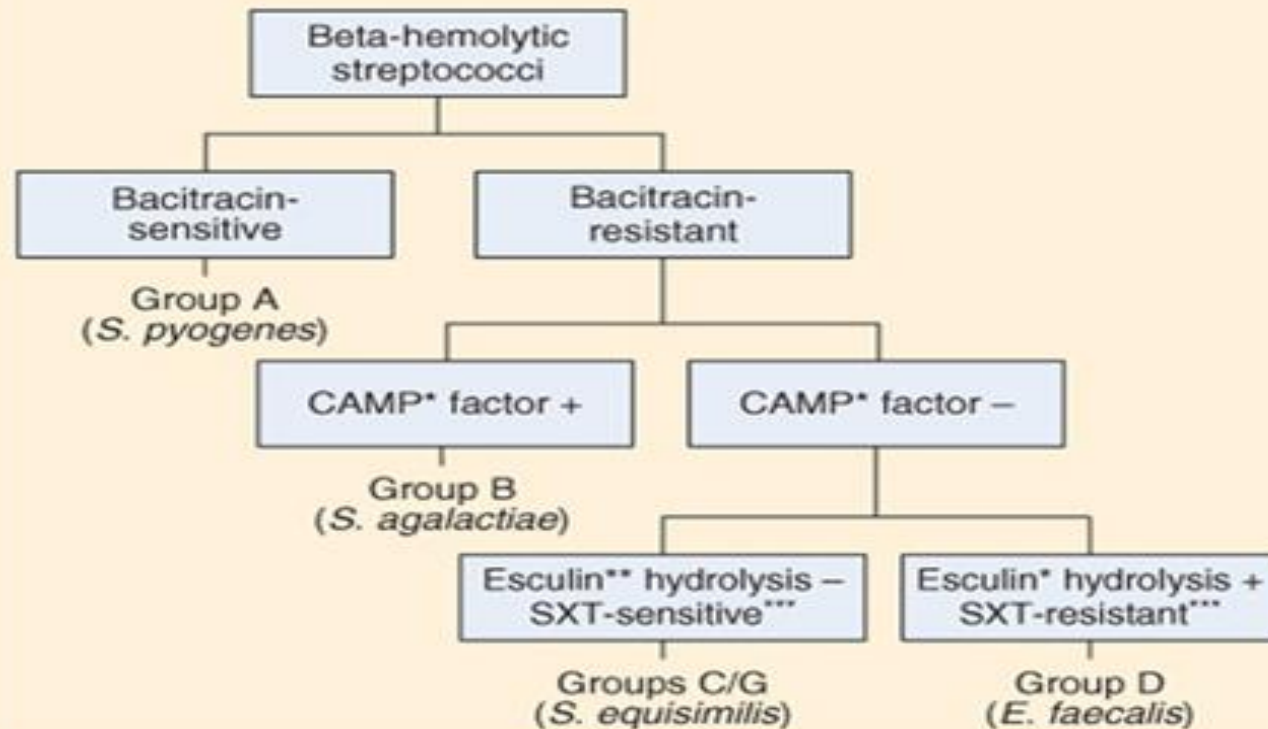


TABLE 18.4**Scheme for Differentiating Beta-Hemolytic Streptococci**

*Name is derived from the first letters of the names of its discoverers. CAMP is a diffusible substance of group B, which lyses sheep red blood cells in the presence of staphylococcal hemolysin.

**A sugar that can be split into glucose and esculetin. Group D streptococci can accomplish this in the presence of 40% bile.

***Sulfa and trimethoprim. The test is performed (like bacitracin) with discs containing this combination drug.

Schema to differentiate Group A and B from other -hemolytic streptococci



Laboratory diagnosis

Group D Streptococci and *Enterococcus* Species

➤ Microscopic morphology

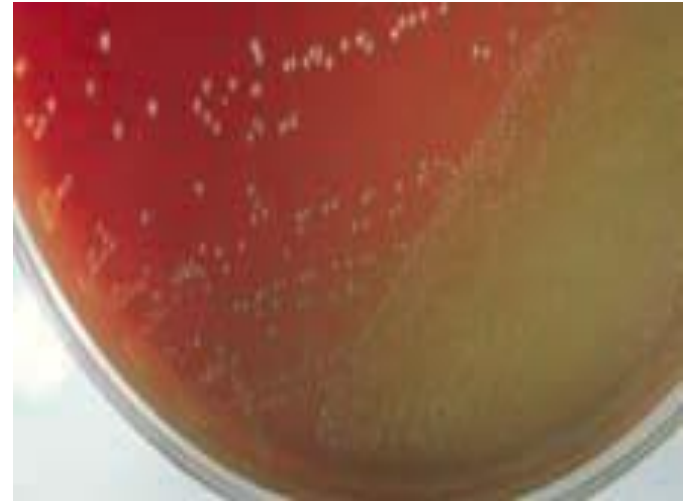
- Cells tend to elongate

➤ Colony morphology

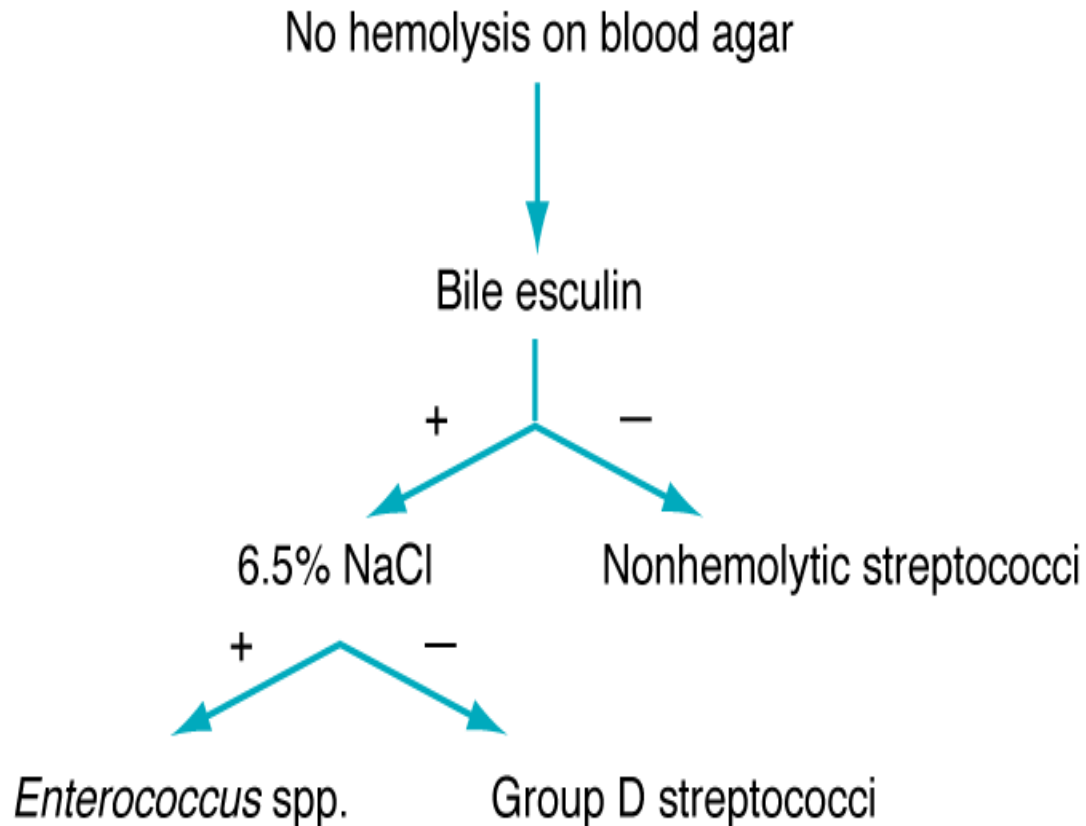
- Most are **non-hemolytic**, although some may show α - or rarely β -hemolysis
- Possess Group **D antigen**

➤ Identification tests

- Catalase: may produce a weak catalase reaction
- Hydrolyze bile esculin
- Differentiate Group D from *Enterococcus* sp. with 6.5% NaCl



Identification Schema



Schema to differentiate *Enterococcus* and Group D streptococci from other non-hemolytic streptococci



Laboratory diagnosis

Streptococcus pneumoniae none groupable

➤ Microscopic morphology

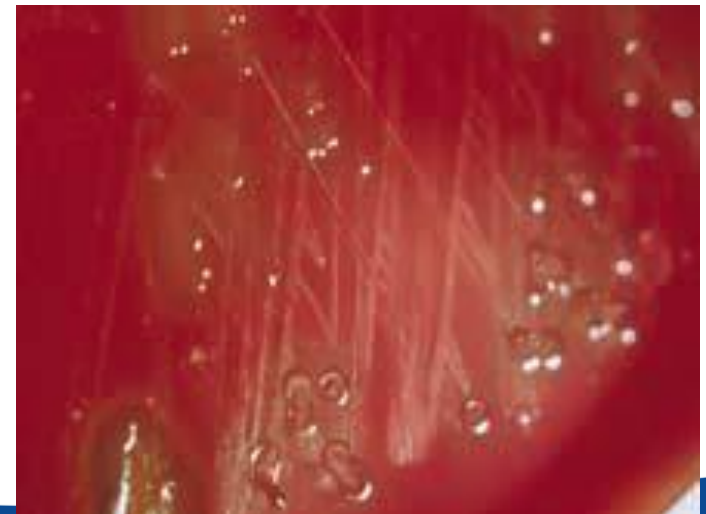
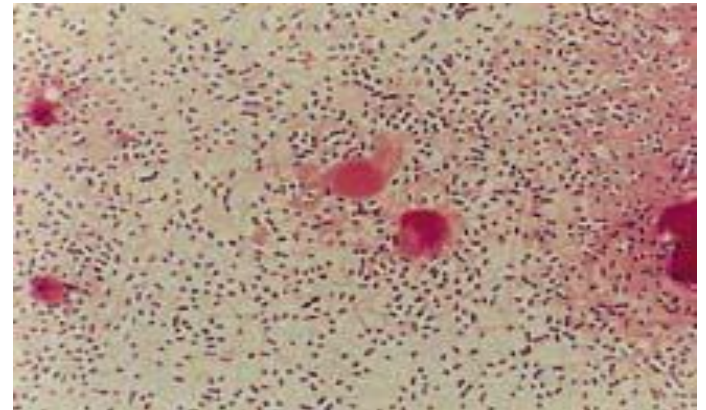
- Gram-positive cocci in pairs; lancet-shaped

➤ Colony morphology

- Smooth, glistening, wet-looking, mucoid, α hemolytic colonies.
- CO₂ enhances growth

➤ Identification tests

- Catalase negative
- Optochin susceptibility test—**susceptible**
- Bile solubility test—**positive**



Viridans streptococci none groupable

- Viridans streptococci are the most prevalent members of the normal microbiota of the upper respiratory tract
- α -hemolytic, but they may also be nonhemolytic.
- Their growth is not inhibited by optochin, and colonies are not soluble in bile (deoxycholate).
- Lack both the group carbohydrate antigens of the pyogenic streptococci and the capsular polysaccharides of the pneumococcus.
- e.g., *S. mutans*

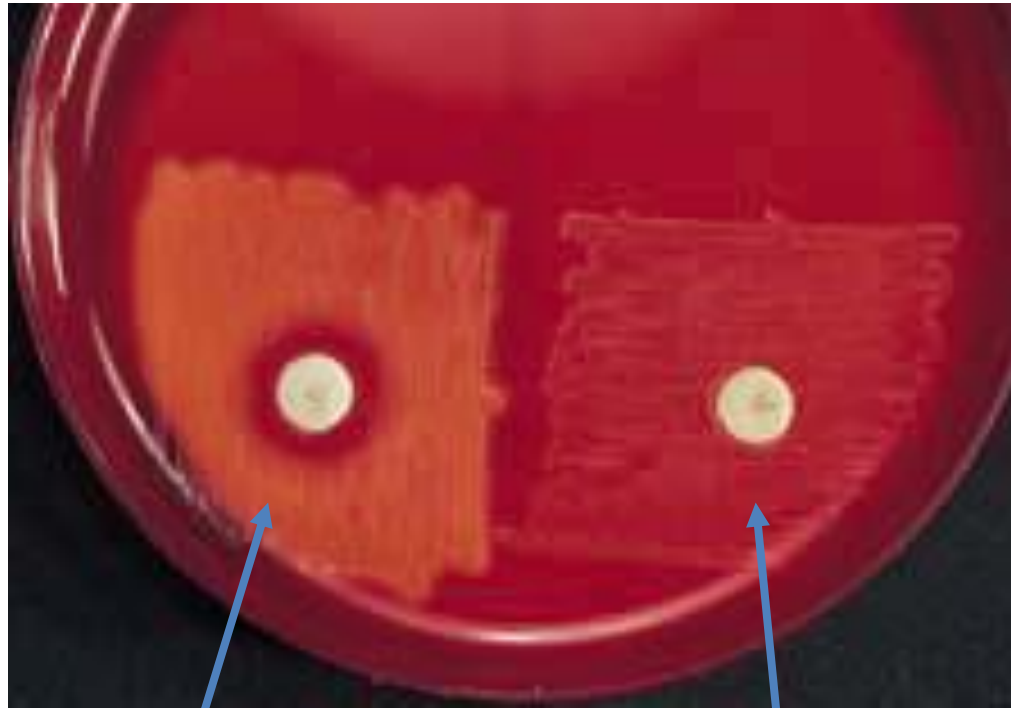


Bacitracin sensitivity test

- **Definitive test** to differentiate between *S. pyogenes* & Non group A β -hemolytic Streptococci (*S. agalactiae*)
- **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:**
 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 37°C for 16-18 hrs.
- **Results:**
 - Positive test: any zone of inhibition around the disc.



Bacitracin sensitivity test



Bacitracin Sensitive

S. pyogenes

Bacitracin Resistant

S. agalactiae



CAMP test

- The **CAMP** phenomenon was first reported in 1944 by **C**ristie, **A**tkins, and **M**unch-**P**eterson.
- Specific for *S. agalactiae* (Group B)

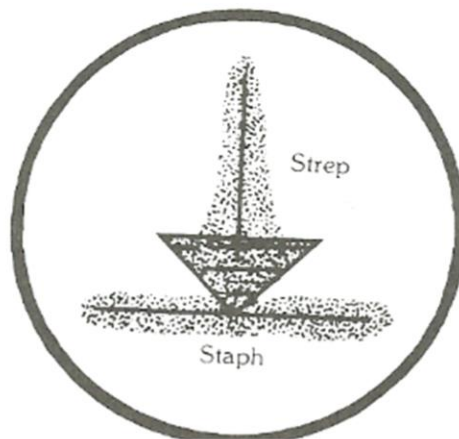
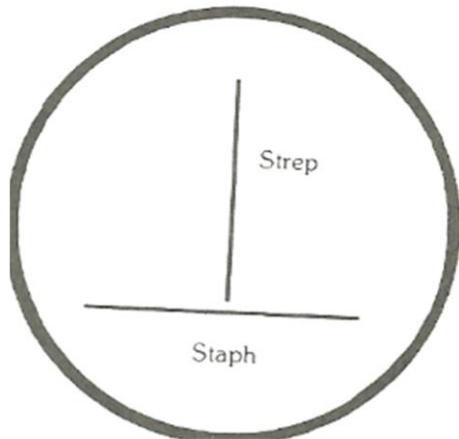
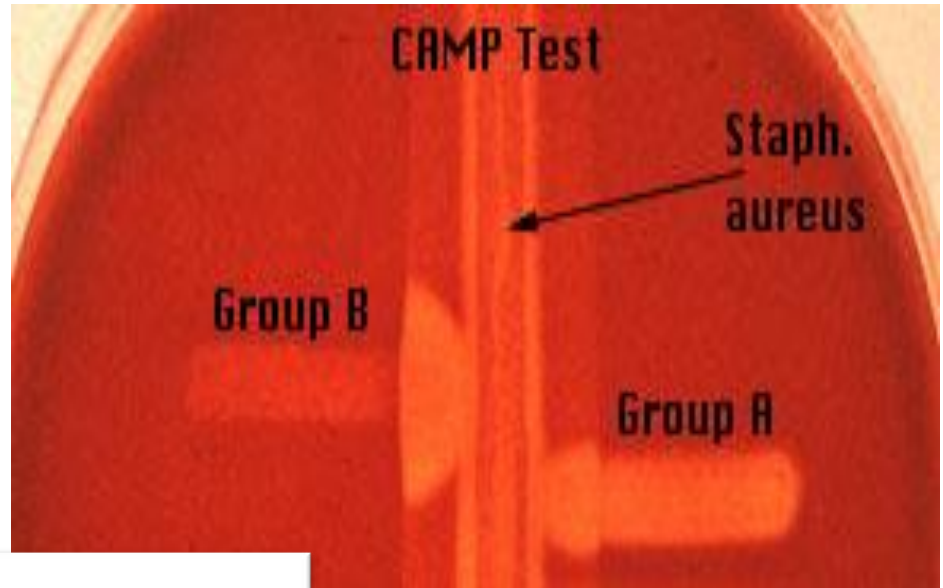
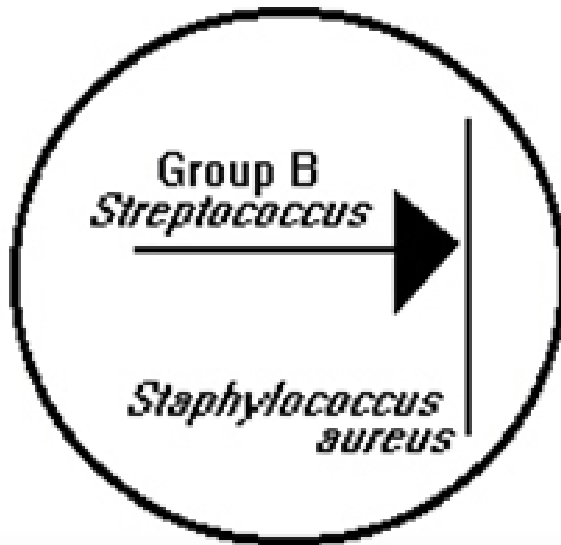
Principle: The hemolytic activity of Staphylococcal beta –lysin on erythrocytes is enhanced by an extracellular factor produced by group B streptococci, called the CAMP factor. Therefore, where the two reactants overlap in a sheep blood agar plate, accentuation of the beta hemolytic reaction occurs.

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 group B streptococci are negative



CAMP test



Optochin sensitivity test

- **Definitive** test to differentiate between *S. pneumoniae* & viridans Streptococci
- **Principle:** *S. pneumoniae* is inhibited by less than 5 µg/ml Optochin reagent (ethylhydroxycupreine hydrochloride) giving a zone of inhibition.
- **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 37° C for 24 hrs.
 4. Accurately measure the diameter of the inhibition zone around the disc.



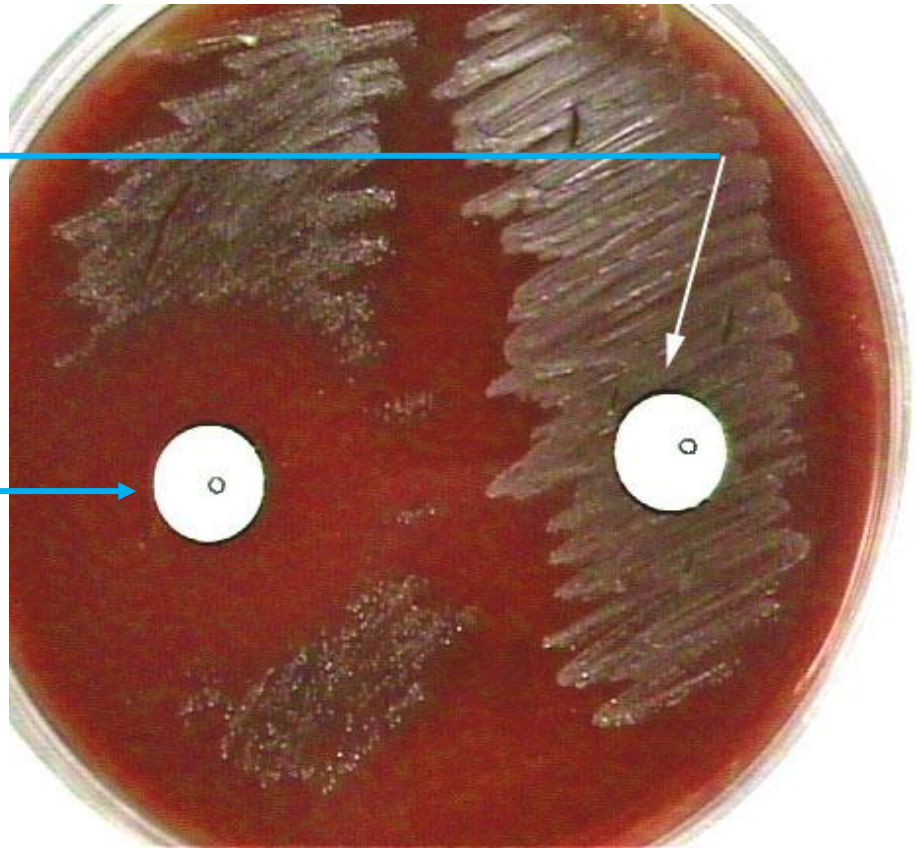
Optochin sensitivity test

Optochin resistant

S. viridans

Optochin susceptible

S. pneumoniae



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>	α	Resistant (≤ 13 mm)	Insoluble	Ferment



Lancefield antigens are cell wall carbohydrates

Presence of Lancefield antigens defines the pyogenic streptococci

Hemolysis is a practical guide to classification

Only pyogenic streptococci are β -hemolytic

Groups A and B streptococci are most common cause of disease

