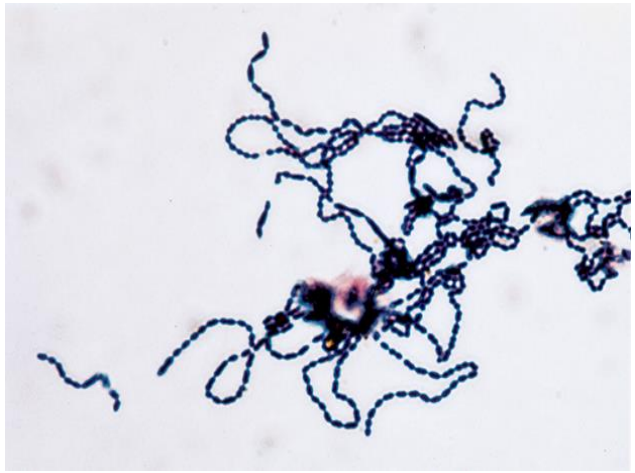
Practical
Medical Bacteriology

Lab 5
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



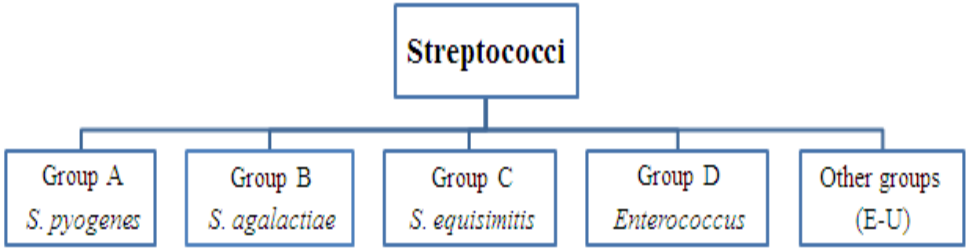
Classification of Streptococci

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*)

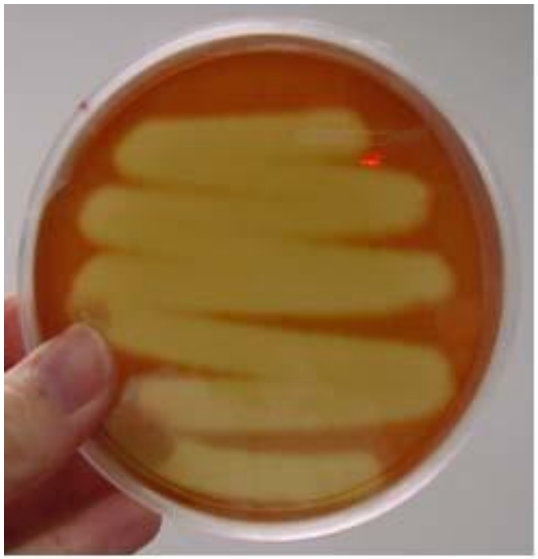
2. β-hemolysis

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

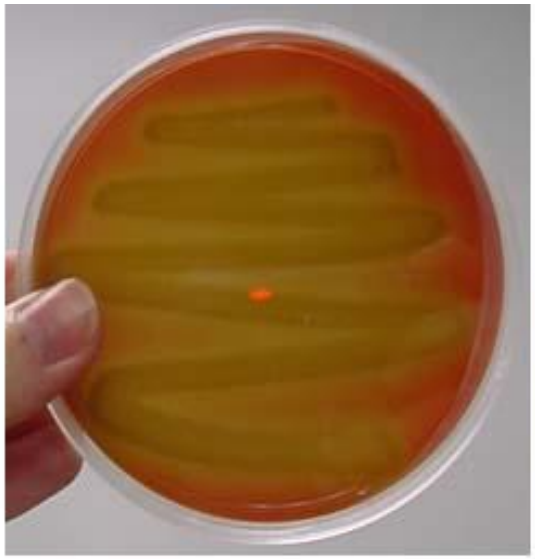
3. γ-hemolysis

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

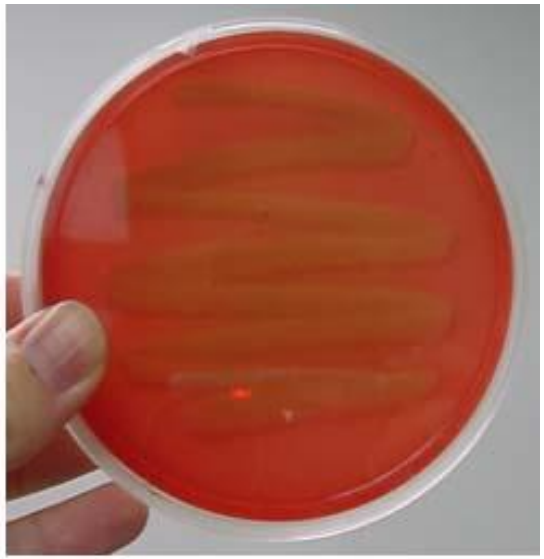
Classification of Streptococci based on hemolysis on blood agar



Beta Hemolysis



Alpha Hemolysis



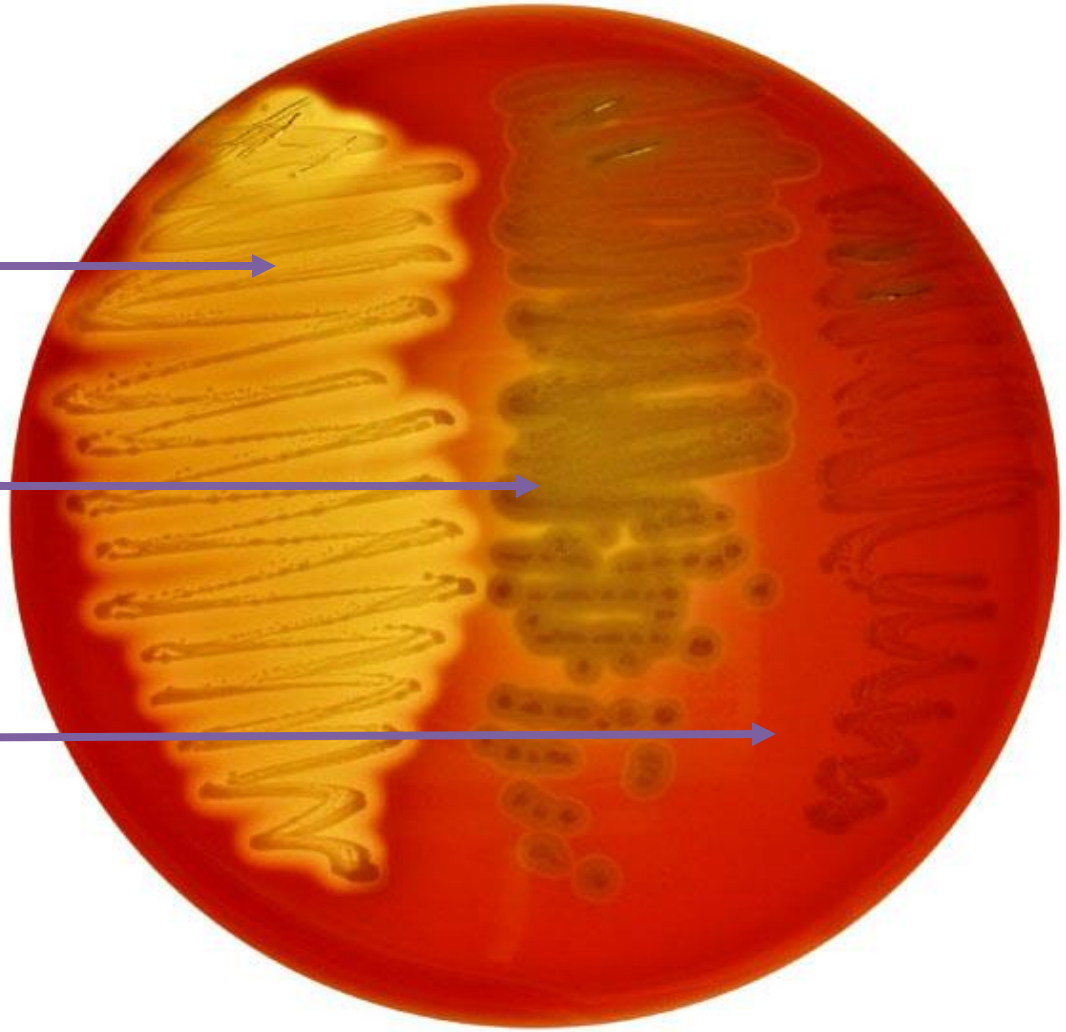
Gamma Hemolysis

Classification of Streptococci based on hemolysis on blood agar

β-hemolysis

α-hemolysis

γ-hemolysis



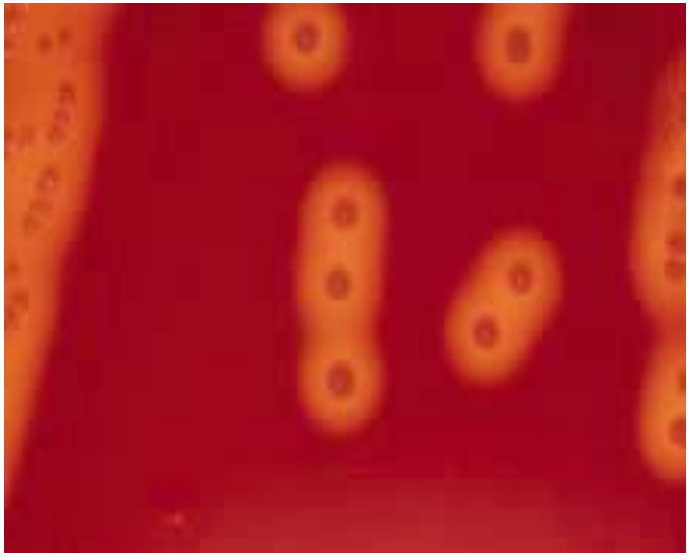
Laboratory diagnosis

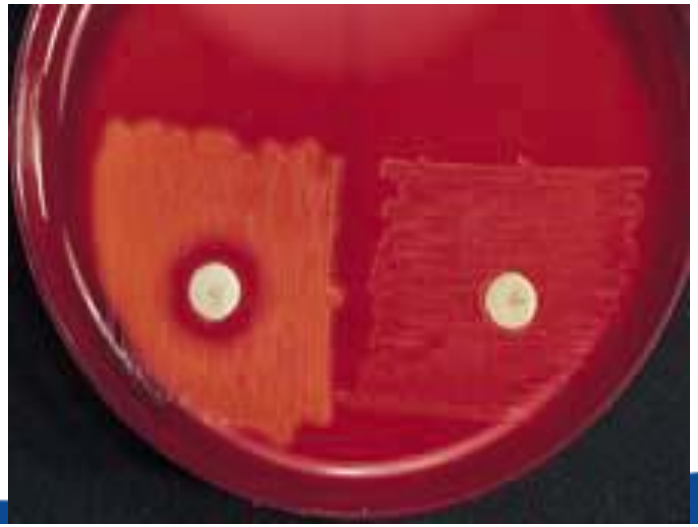
1. **Specimen:**
   - **Throat swab, Pus swab, CSF, Blood, Urine**
2. **Culture:**
   - **Blood agar**
   - **Chocolate agar**
   - **Other selective media**
3. **Biochemical tests**
4. **Serological methods for identification of some strains or species**
5. **Antibiotic sensitivity tests**
6. **Molecular 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



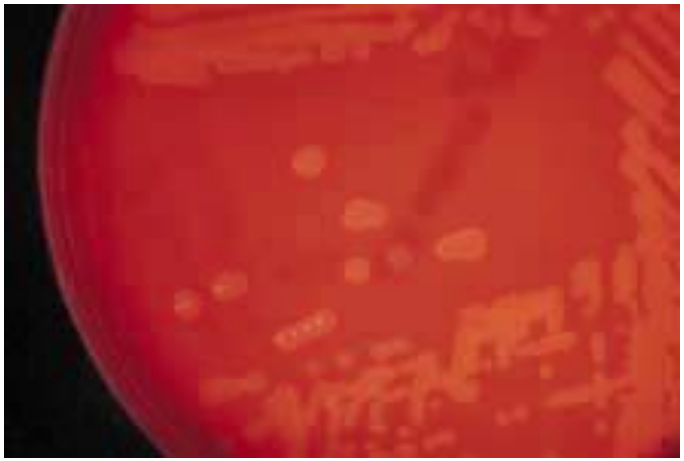


- Group A streptococci (*S. pyogenes*) is susceptible to Bacitracin disk (left); The right shows resistance

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



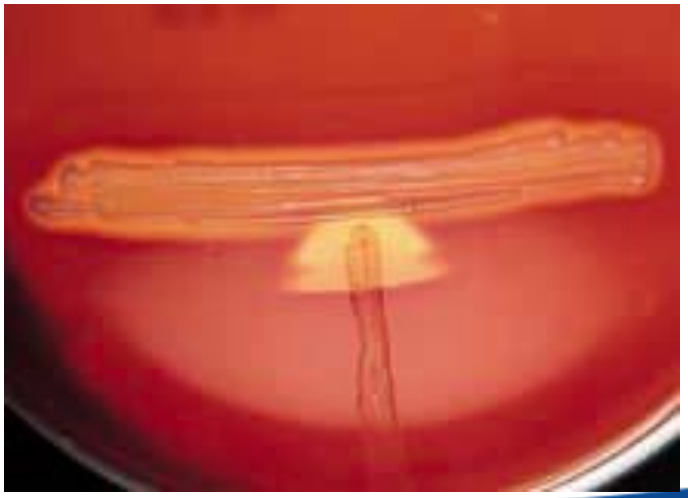
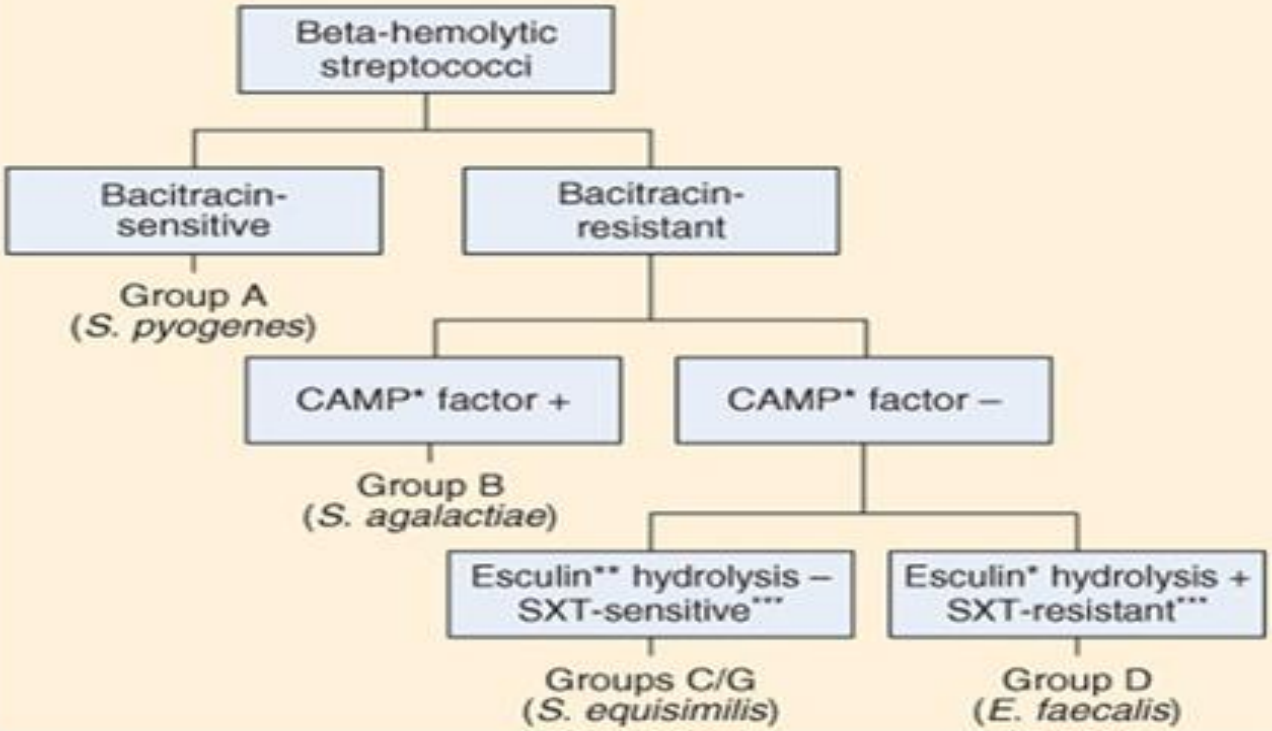


TABLE 18.4 Scheme for Differentiating Beta-Hemolytic Streptococci



*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.

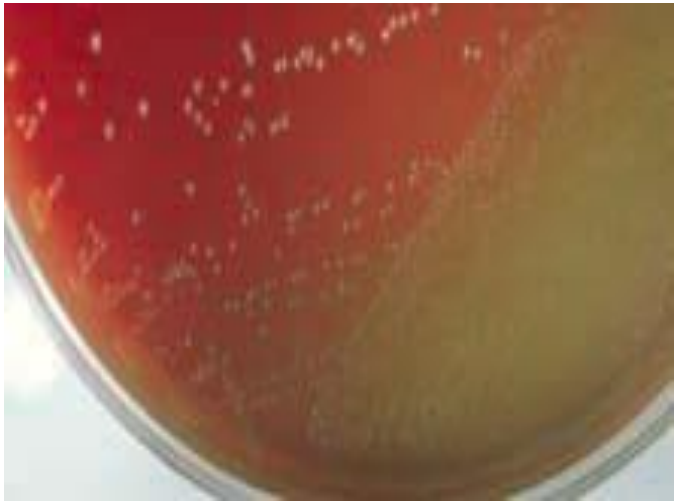
***Sulfa and trimethoprim. The test is performed (like bacitracin) with discs containing this combination drug.

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

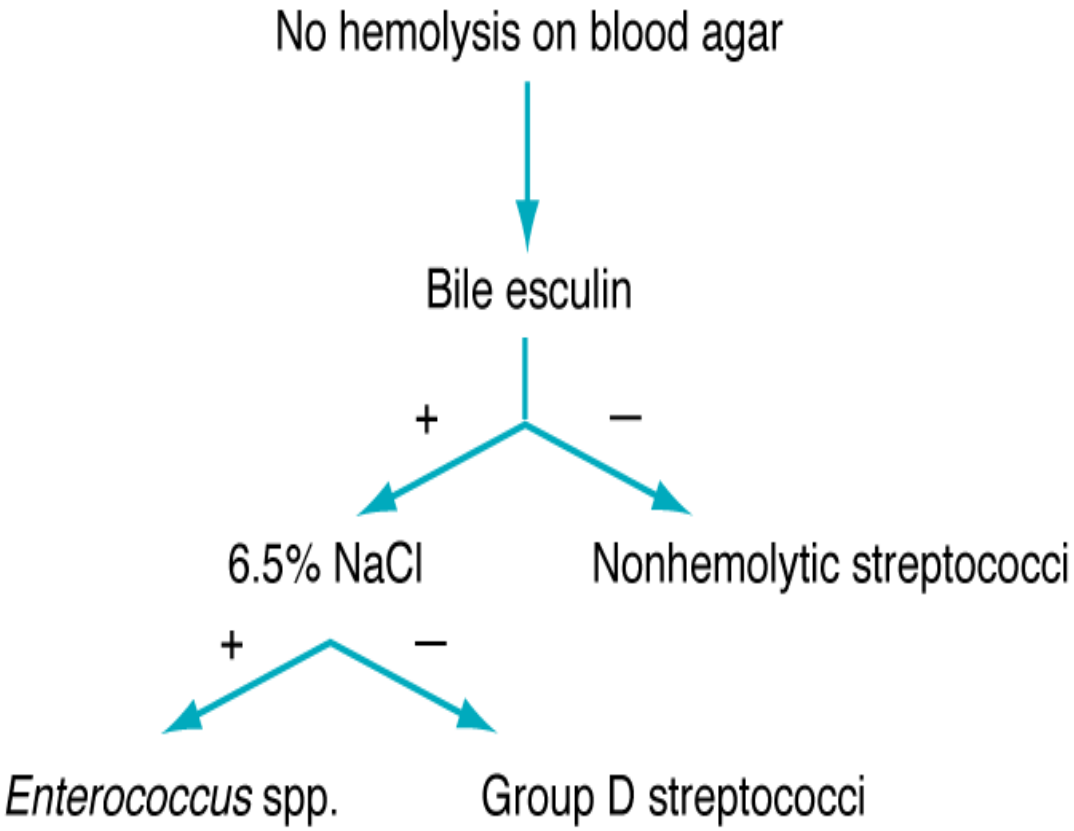
Laboratory diagnosis

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

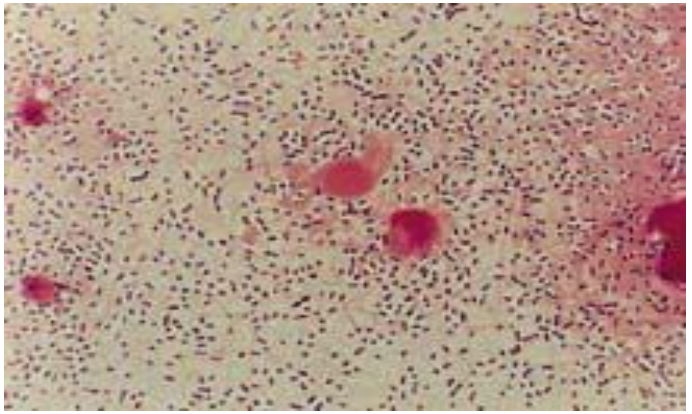


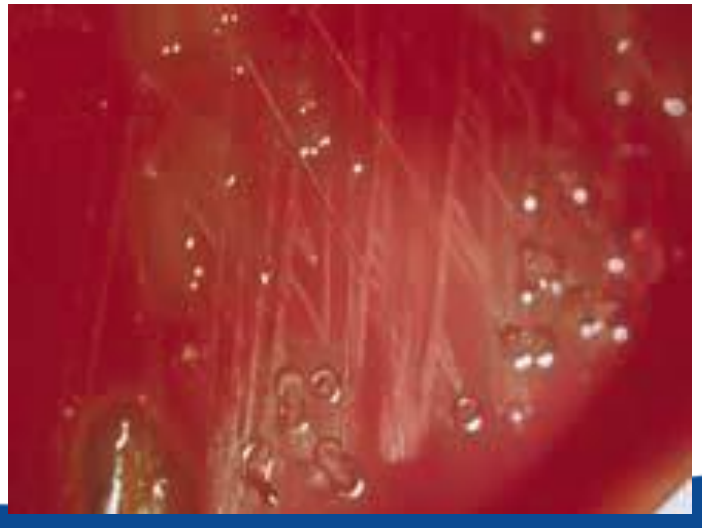
Schema to differentiate Enterococcus and Group D streptococci from other non-hemolytic streptococci

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.
  - CO_2 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

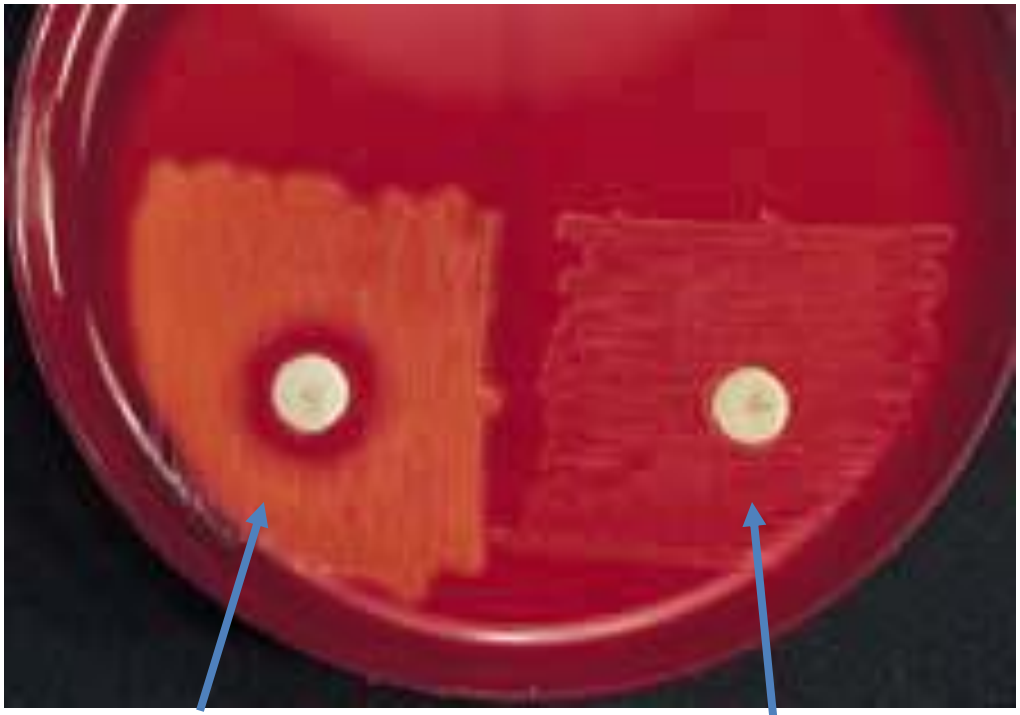
- **Definitive test** to differentiate between ***S. pyogenes*** & Non group A β-hemolytic Streptococci (*S. agalactiae*)

- **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 37oC 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

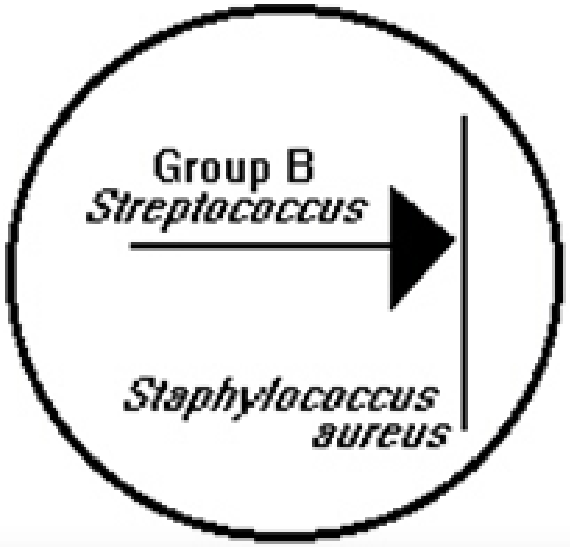
- The CAMP phenomenon was first reported in 1944 by **C**ristie, **A**tkins, and **M**unch-**P**eterson.
- Specific for ***S. agalactiae*** (Group B)

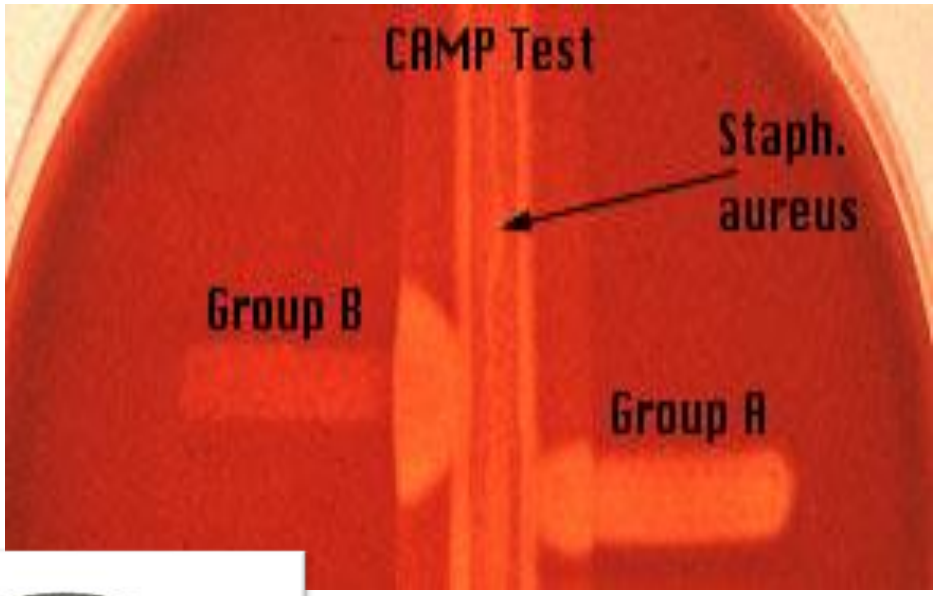
Principle: The hemolytic activity of Staphylococcal beta –lysin on erythrocytes is enhanced by an extracellular factor produced by group B streptococci, called the CAMP factor. Therefore, where the two reactants overlap in a sheep blood agar plate, accentuation of the beta hemolytic reaction occurs.

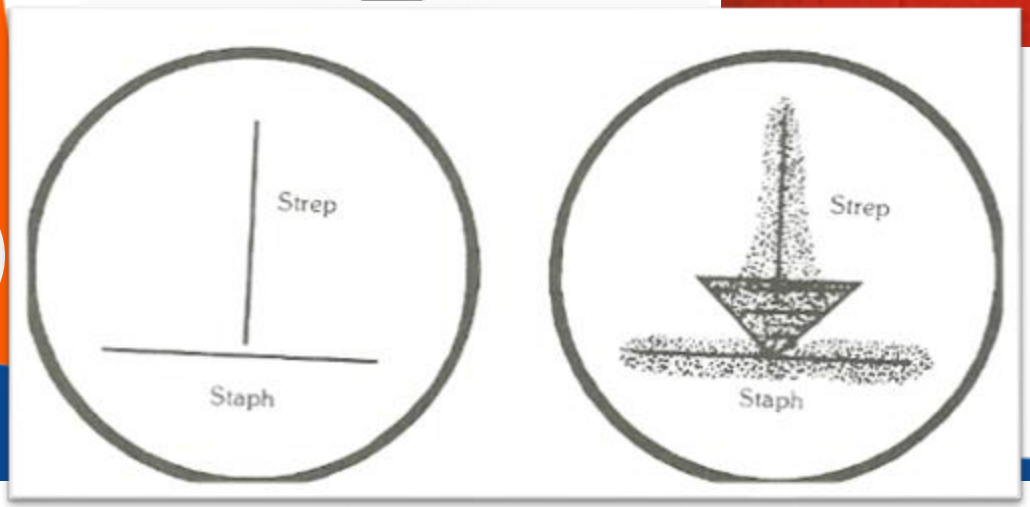
Procedure:

- 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


Group B
Streptococcus
Staphylococcus
aureus


CAMP Test
Staph.
aureus
Group B
Group A


Strep
Staph
Strep
Staph

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.

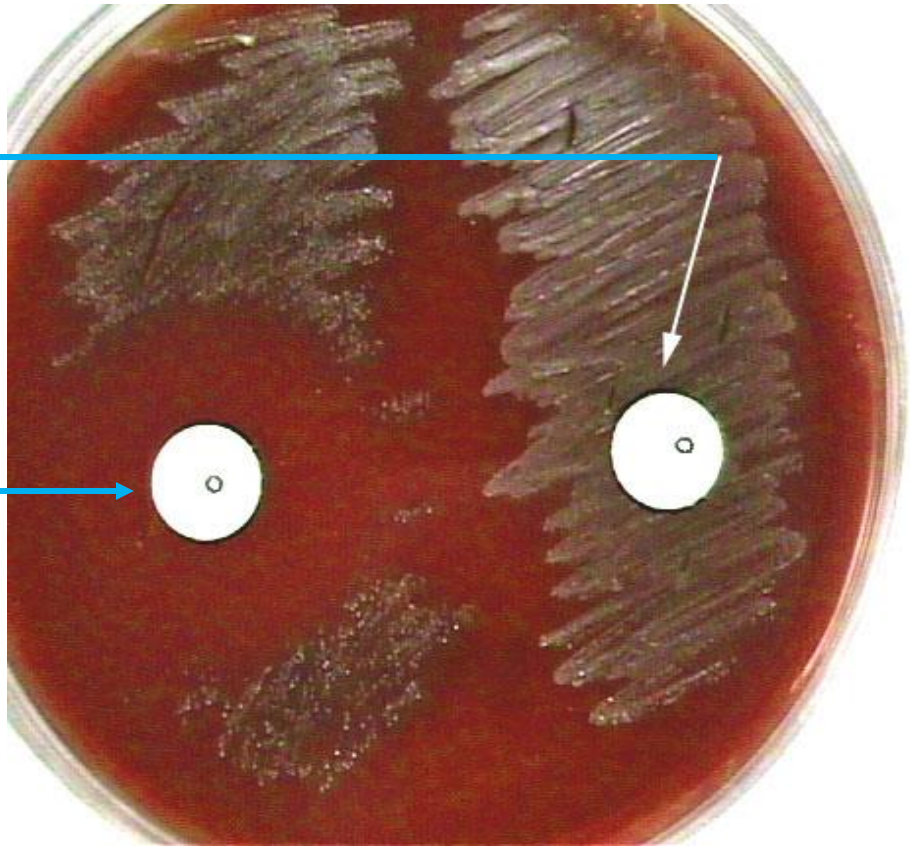
Optochin sensitivity test

Optochin resistant

S. viridans

Optochin susceptible

S. pneumoniae



Differentiation between β-hemolytic streptococci

	Hemolysis	Bacitracin sensitivity	CAMP test
S. pyogenes	β	Susceptible	Negative
S. agalactiae	β	Resistant	Positive

Differentiation between α-hemolytic Streptococci

	Hemolysis	Optochin sensitivity	Bile solubility	Inulin Fermentation
S. pneumoniae	α	Sensitive (≥ 14 mm)	Soluble	Not ferment
Viridans strep	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