

# *Pathogenic Bacteria* *( practical )*



## Family: Micrococcaceae

### Genus: Streptococcus

*S. pyogenes*

*S. agalactiae*

*S. equi*

*S. bovis*

*S. salvarius*

*S. mitis*

*S. mutans*

*S.pneumonia*

### Family: Enterococcaceae

#### Genus: *Enterococcus faecalis*

**General Characteristics:** The streptococci are gram-positive cocci arranged in chains; however, as a culture ages and the bacteria die, they lose their gram-positivity and can appear to be gram-negative, this can occur after overnight incubation. Catalase and oxidase negative. They are widely distributed in nature. Some are members of the normal human flora. Several species of streptococci are normal inhabitants of the pharynx, they can also be isolated from surfaces of the teeth, the saliva, skin, colon, rectum, and vagina. Others are associated with important human diseases attributable in part to infection by streptococci. The pneumococci (*S. pneumoniae*) are diplococci, often lancet-shaped or arranged in chains, possessing a capsule of polysaccharide that permits typing with specific antisera. Pneumococci are readily lysed by surface-active agents, which probably remove or inactivate the inhibitors of cell wall autolysins.

**Culture and Growth Characteristics:** The colonies on the blood agar are circular, small, semi-translucent gray-white colonies. Growth of streptococci tends to be poor on solid media or in broth unless enriched with blood or tissue fluids. Nutritive requirements vary widely among different species. Growth and hemolysis are aided by incubation in 10% CO<sub>2</sub>. Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

**Classification and Pathogenesis:** Streptococci are classified according to:

1. Their haemolytic activity (type of haemolysis  $\alpha$ ,  $\beta$ ,  $\gamma$ )
2. Immunologic properties (the serological classification of Lancefield) serologic specificity of the cell wall group-specific substance and other cell wall or capsular antigens. According to this classification there are many serogroup associated with human disease that are groups A, B, C & D.
3. Resistance to chemical and physical factors.

4. Ecologic features: Molecular genetics have also been used to study the Streptococci.

**Specimens:** Specimens to be obtained depend upon the nature of the streptococcal infection. A throat swab, pus, or blood is obtained for culture. Serum is obtained for antibody determinations. CSF and sputum are collected for demonstration of pneumococci.

**Laboratory diagnostic tests:**

1. **Gram stain** (gram-positive cocci)
2. **Blood agar** ( for type haemolysis)
3. **Carbohydrates fermentation** (glucose, inulin, mannitol & lactose)
4. **Bile solubility: in the** presence of bile salts (Na-deoxycholate or Na-taurocholate), the surface tension of this salts cause release of autolytic enzyme that cause lysis of the cells.

Positive result: cell lysis (no growth).

Negative result: growth of cell.

5. **Streptokinase test:** Streptokinase transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins. Indicator for this test is the lysis of the plasma clot (inverse coagulase), to make a clot in plasma  $\text{CaCl}_2$  was added to the plasma then the growth of bacteria were added to see the Streptokinase production.

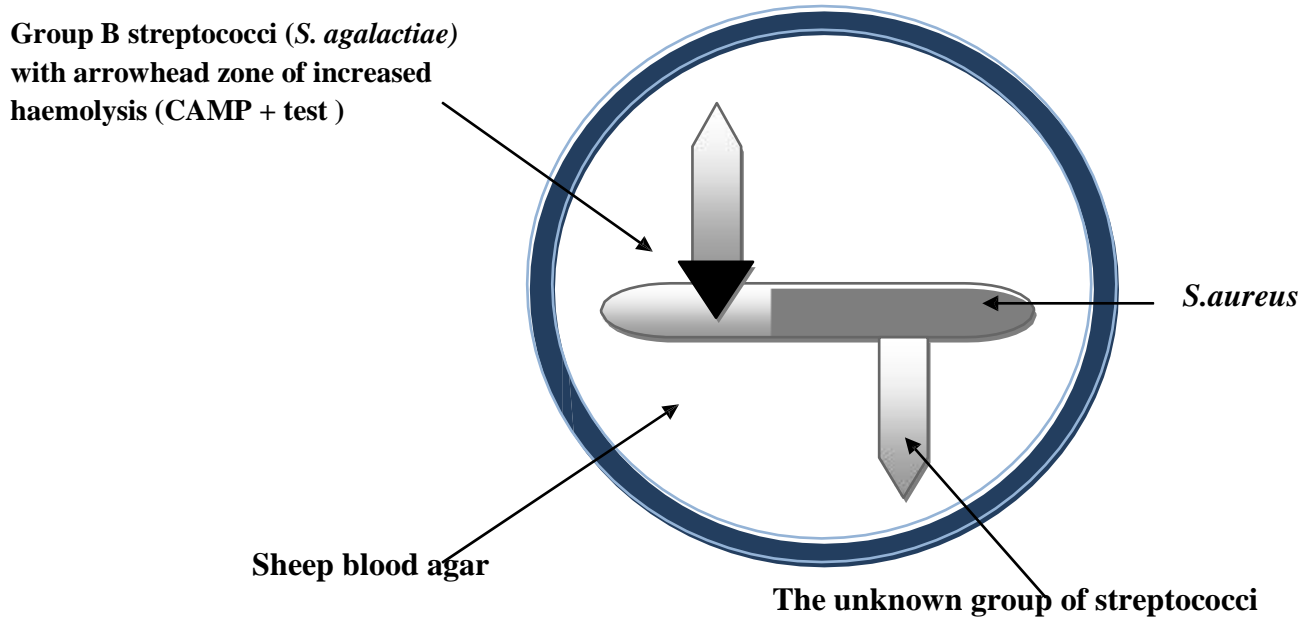
Positive result: no clot

Negative result: clot

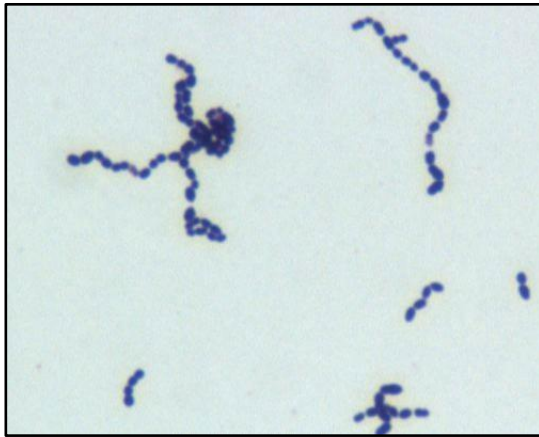
All species of streptococcus produce Streptokinase except *S. pneumoniae*

6. **Bacitracin and Optochin Susceptibility:** *S. pyogenes* can be distinguished from viridans streptococci by means of the **bacitracin antibiotic disk** (*S. pyogenes* inhibited by Bacitracin). **Optochin disks** are used for differentiation of the alpha hemolytic viridans streptococci from the pneumococci. The pneumococci are sensitive to these disks; the viridans organisms are resistant.
7. **Quelling reaction "capsule swelling test":** this test is performed for capsulated *S. pneumoniae*. When pneumococci are mixed with specific antiserum (anti-capsular Ag) on a microscope slide, the capsule swells, and the organisms agglutinate by cross-linking of the antibodies, indicating the positive result
8. **Salt Tolerance (6.5% NaCl):** All enterococci of group D produce heavy growth in 6.5% NaCl broth, none of the nonenterococci, group D, grow in this medium. This test, then, provides us with a good method for differentiating the two types of group D streptococci.
9. **CAMP Reaction:** Group B streptococci (*S. agalactiae*) can be distinguished from other  $\beta$ -hemolytic streptococci by their production of a substance called the CAMP factor. This factor is a peptide that acts together with the  $\beta$ -hemolysin produced by some strains of *S. aureus*, enhancing the effect of the  $\beta$ -hemolysin on a sheep blood agar plate. With an inoculating loop, streak a strain of *S. aureus* down the centre of a blood agar plate. On one side of the plate, inoculate a standard strain of Group B streptococci (*S. agalactiae*) by making a streak at a  $90^\circ$  angle, starting 5 mm away from the *S. aureus* and extending outward to the edge of the agar. On the other side of the plate, inoculate the unknown strain of streptococci. This streak should not be directly opposite the Group B streak. Incubate the plate at  $35^\circ\text{C}$  for

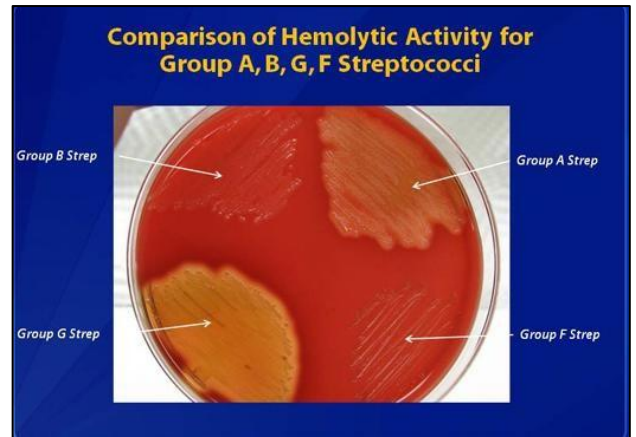
24 hours. Haemolysis of *S.aureus* interact with haemolysis of Group B streptococci (*S.agalactiae*) to form a flame shape or arrow head zone of increased haemolysis between the two genera, other groups of *Streptococcus* spp. does not show this shape.



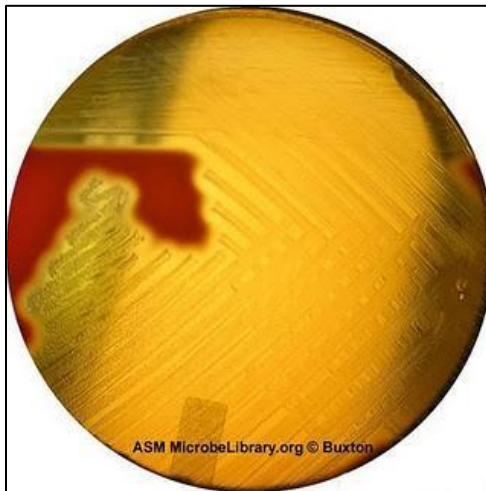
Test	<i>S. pneumoniae</i>	<i>S. salvarius</i>	<i>Enterococcus faecalis</i>	<i>S. pyogenes</i>
Inulin	+	+/-	-	-
Lactose	-	+	+	+ no gas
Mannitol	-	-	+	+ no gas
Glucose	-	+	+	+ no gas
Bile salt solubility	+ no growth	- growth	- growth	- growth
Optochin	+ no growth	- growth	- growth	- growth
Bacitracin	- growth	- growth	- growth	+ no growth
CAMP reaction	-	-	-	-
Haemolysis	$\alpha$	$\alpha$	$\alpha$	$\beta$
Growth at 6.5% NaCl	- no growth	- no growth	+ growth	+/-
Streptokinase	- clot	+ no clot	+ no clot	+ no clot



Streptococci gram-positive cocci arranged in chains



Types of blood haemolysis



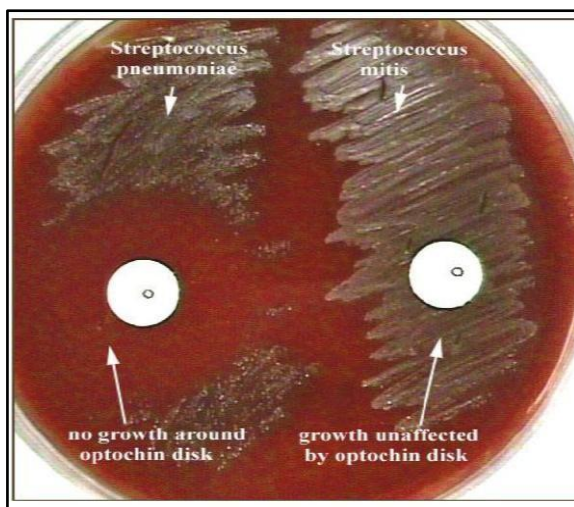
*S. pyogenes*



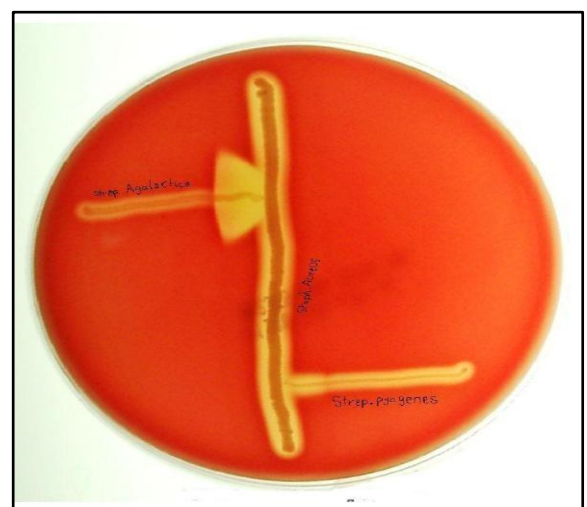
*S.pneumoniae*



Viridans streptococci



Optochin Susceptibility



CAMP Reaction

