LAB 5:

GENUS: STREPTOCOCCUS

Taxonomic Classification:

Kingdom: Bacteria

Phylum: Firmicutes

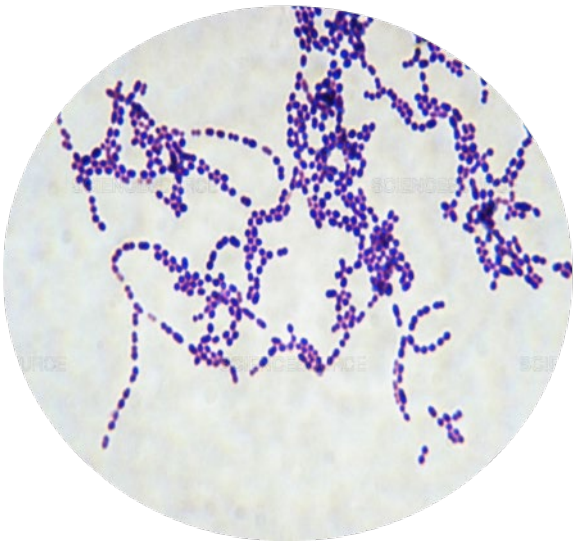
Class: Bacilli

Order: Lactobacillales

Family: Streptococcaceae

Genus: *Streptococcus*

General characteristics:



Gram positive, cocci, arranged in chain or in pairs, non-spore forming, non-motile, anaerobic to facultative anaerobe, fastidious, catalase negative, the presence of CO_2 enhance the growth as well as humidity, colonies appear as a very small in blood agar imbedded in the agar like pin point. sometime appear in gram stain as a Gram negative rod, this variation in shape depend on age of culture (Due to the autolysis of the cells).

Diseases caused by *Streptococcus*

Group	Hemolysis type	*Streptococcus* spp.	Diseases
A	*β*	*Strept. pyogenes*	Tonsillitis, Bronchopneumonia, Scarlet fever, Cellulites, Glomerulonephritis, Rheumatic fever
B	*β*	*Strept. Agalatiae* (CAMP +ve)	Neonatal endocarditis and meningitis
C	*β*	*Strept. equisimilis*	Throat infection, Puerperal fever
D	*α*	*Strept. viridans*	Subacute endocarditis to heart failure
	α	*Strept. pneumoniae (Optochin +ve)*	pneumonia

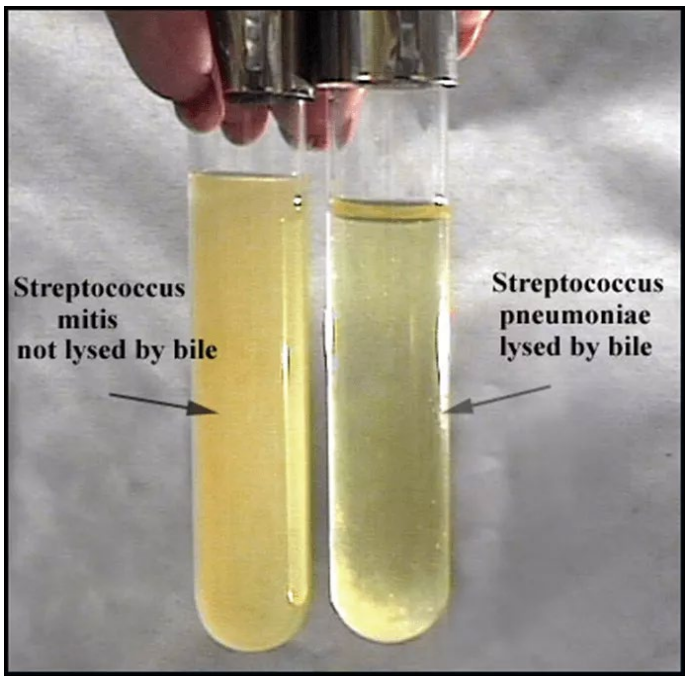
***Specimens:** Throat swab, Sputum, Pus, Blood for culture, Serum for antibodies determination.

Lab. Diagnostic tests:

1- **Gram stain:** G+ ve cocci

2- **Blood agar** for hemolysis detection.

3- **Bile solubility test:** The principle of the bile (sodium deoxycholate or sodium taurocholate) solubility test is to distinguishes *Strepto. pneumoniae* from all other alpha-hemolytic streptococci. *Strepto. pneumoniae* is bile soluble whereas all other alpha-hemolytic streptococci are bile resistant. Sodium deoxycholate (2% in water) will lyse the pneumococcal cell wall. *Strepto. pneumoniae* produces autolytic enzymes (autolysins) that cause lysis of older cells. These autolysins are responsible for the sunken centers that are observed in older *Strepto. pneumoniae* colonies on agar media. The bile solubility test uses bile salts to accelerate the lytic process.



Interpretation of results: Visible clearing of the suspension in the "test" tube and no change in the turbidity of the suspension in the control tube constitute a positive test result. No change in the turbidity of the suspension in the "test" tube relative to the turbidity in the control tube constitutes a negative test result. Most strains of *S. pneumoniae* are bile solubility positive.

4- Streptokinase test: Kinases (also known as fibrinolysins) have the opposite effect of coagulase. Streptokinase is the name of a kinase produced by streptococci. Thus, the positive indication of this test is the lysis of the plasma clot.

In addition to the Coagulase test the Streptokinase teat help to differentiate Streptococcus from Staphylococcus.

*All species of *Streptococcus* are producing streptokinase except *Strept. pneumoniae* (-)

To make the test prepper a clot from plasma by adding Cacl2 then add bacterial growth to see streptokinase production.

*Clot test = 0.2 ml human plasma + 0.25 (Cacl2) + 0.8 saline + 0.5 ml culture

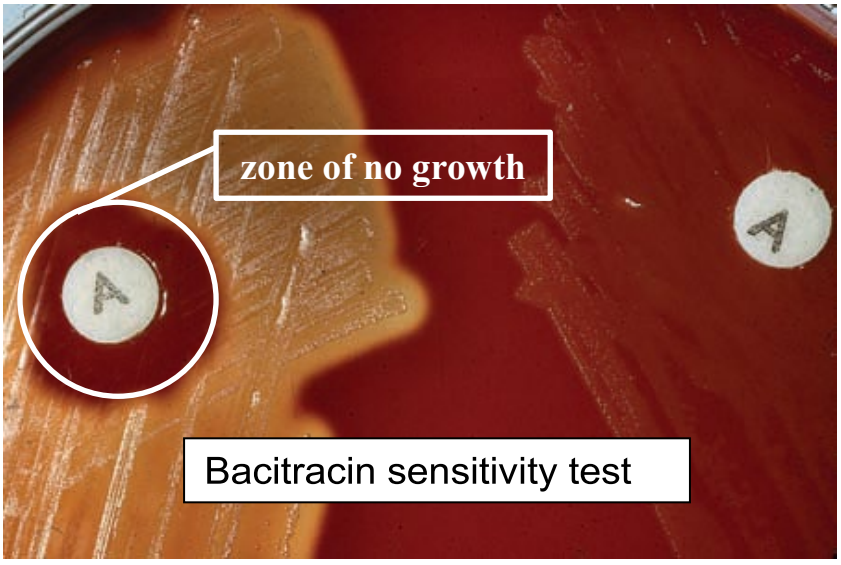
*Control = 0.2 ml human plasma + 0.25 (Cacl2) + 0.8 saline + 0.5 ml saline.

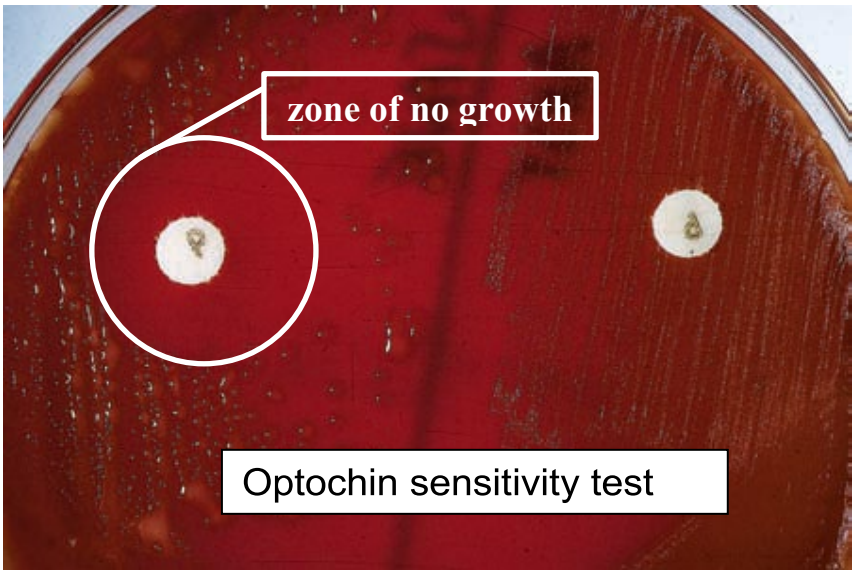
5- Carbohydrate fermentation (To differentiate between *Strept. spp.*).
Because *Strept.* are fastidious a Muller-Hinton medium with CHO is used for fermentation, the indicator is bromothymol blue and the sugars that used will be glucose, inulin, mannitol and lactose.

6- Bacitracin and Optochin test:

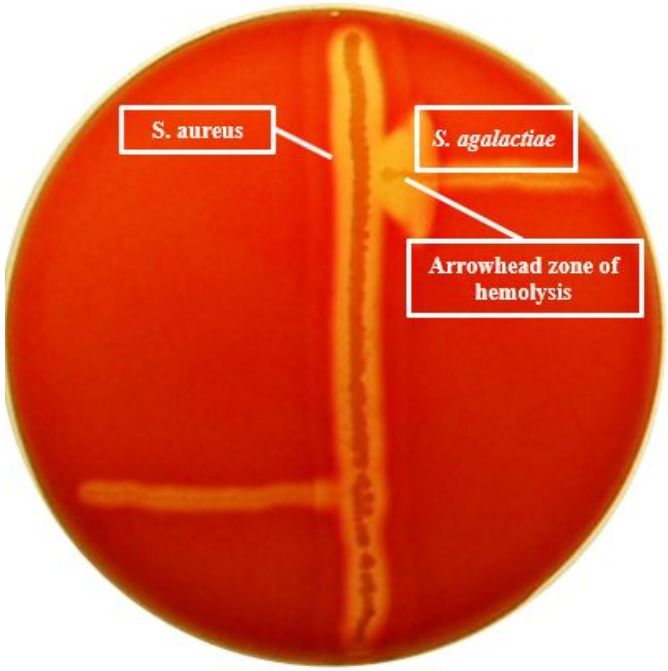
The traditional method of differentiating *S. pyogenes* from other α-hemolytic Streptococcus spp. of human origin was the A-disk or bacitracin sensitivity test and any zone of inhibition of growth is considered as a positive test result. (The A in A-disk refers to group A Strep.) Positive and negative A-disk test results are shown in figure below.

The traditional method of differentiating *S. pneumoniae* from other α-hemolytic Streptococcus spp. of human origin is the P-disk (Optochin sensitivity) test. The P in P-disk stands for pneumoniae. In this test, an Optochin impregnated paper disk is placed onto the surface of a blood agar plate that has previously been heavily inoculated with isolates. Optochin kills *S. pneumoniae* but does not kill other α-hemolytic streptococci. Following overnight incubation of the inoculated plate at 35ºC in a CO2 incubator, the plate is examined for a zone of no growth around the Optochin disk. If the zone of no growth is 14 mm (using a 6-mm-diameter disk) or 16 mm (using a 10-mm-diameter disk), the organism can be identified as *S. pneumoniae* (see figure below). If the isolate produces a smaller zone of no growth, then the isolate should be tested for bile solubility.





7- CAMP factor: The initials CAMP is taken from the last names of each of the three people who developed the original CAMP test: Christie, Atkins, and Munch-Peterson. The CAMP factor is a diffusible extracellular protein that acts synergistically with Staphylococcal β-lysin to cause enhanced lysis of red blood cells. A blood agar plate is inoculated by making perpendicular streaks of a β-lysin producing strain of *S. aureus* and the organism to be tested (see figure below). The streaks do not touch each other but are about 3 to 4 mm apart. Incubation is done overnight at 35˚C. An arrowhead-shaped zone of enhanced hemolysis in the area into which both the β-lysin and the CAMP factor have diffused represents a positive test result.



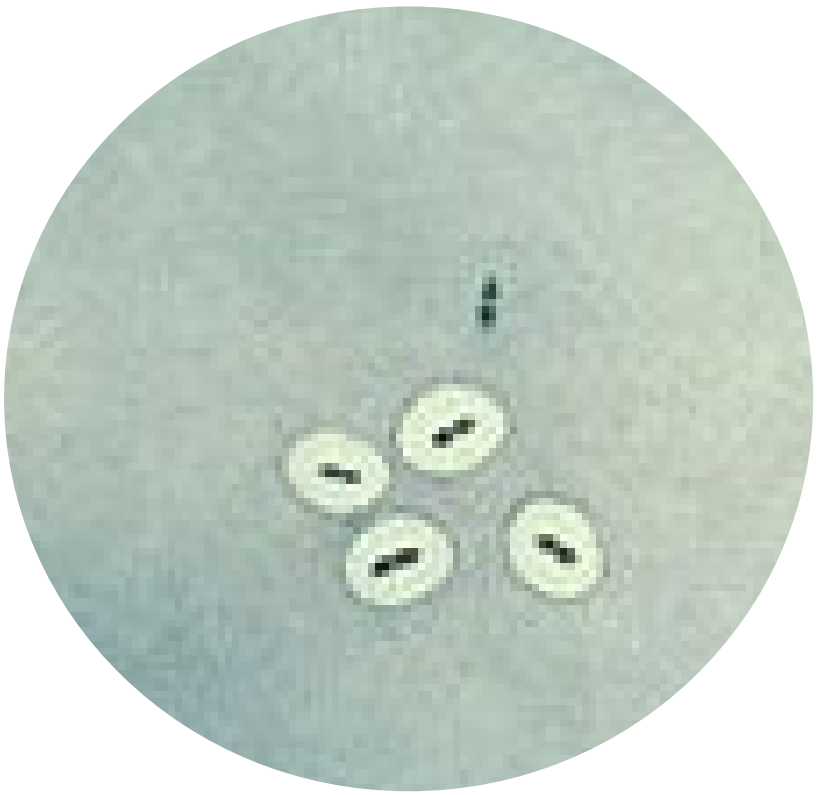
S. agalactiae gives a positive test result.

The term enhanced hemolysis refers to an area of more complete β-hemolysis than is produced by either the β-lysin or CAMP factor alone.

8- Growth in 6.5 % Na cl: The salt tolerance test is performed using Tryptic Soy Broth with added sodium chloride (regular table salt) to create an overall salt concentration of 6.5%. It is a selective medium which tests the ability of an organism to survive in a salt-rich environment. (+ve) turbidity and (-ve) where there is no growth.

9- Quelling reaction: also called the Neufeld reaction, is a biochemical reaction in which antibodies bind to the bacterial capsule of *Streptococcus pneumoniae.* The antibody reaction allows these species to be visualized under a microscope. If the reaction is positive, the capsule becomes opaque and appears to enlarge (see figure below)

The test is performed by mixing bacterial growth with standard specific anti-capsular Ag. swelling of the capsule indicates the (+ve) result.



* Some *Streptococcus* spp. features:

Test	*Strept pneumoniae*	*Strept. viridans*	*Strept. pyogenes*
Inulin	+	+\-	-
Lactose	-	+	+ no gas
Mannitol	-	-	+ no gas
Glucose	-	+	+ no gas
Bile solubility	(+) no growth	(-) growth	(-) growth
Optochin	+(S)	- (R)	-
Bacitracin	-	-	+
CAMP	-	-	-
Hemolysis	α	α	ß
Growth at 6.5% Nacl	(-) no growth	(-) no growth	+\-
Streptokinase	-	+	+