

The isolation and identification of Streptococcus

Lab. second course

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General properties :

Is a genus of coccus (spherical) , They are **catalase-negative, Gram-positive spherical** or oval cocci in pairs and chains .

Streptococci can be classified according to:

- Oxygen requirements
- Hemolysis on blood agar

Sterptococci are divided into three main groups according to hemolytic reactions on blood agar α-hemolytic cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar Sterptococci. Beta-hemolytic species cause complete rupture of red blood cells on blood agar and Gamma-hemolytic species cause no hemolysis.

- **Serology (Lanciefield Classification)** The carbohydrate antigens found on the cell walls of the organisms are related to their virulence.
- ■ **group A** includes the important human pathogen *Streptococcus pyogenes*
- ■ **group B** contains one species, *Streptococcus agalactiae*,; it causes infection in neonates
- ■ **group C** mainly causes diseases in animals
- ■ **group D** includes the enterococci (*Enterococcus faecalis*, an endodontic pathogen) .
- In the medical, the most important groups are the **alpha-hemolytic streptococci Streptococcus pneumonia** and **S. viridians group**, and the beta-hemolytic streptococci of Lancefield groups A and B Streptococci are oxidase- and catalase-negative, and many are facultative anaerobes

Laboratory diagnosis :

1. Gram Stain (positive)(Fig.1)

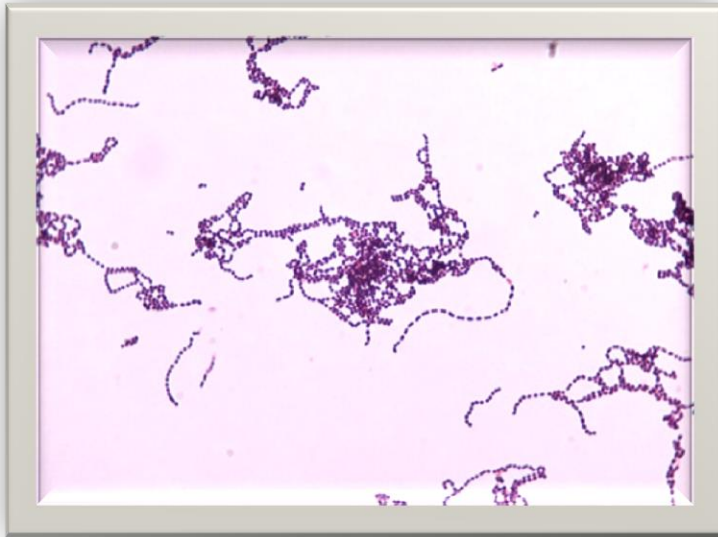
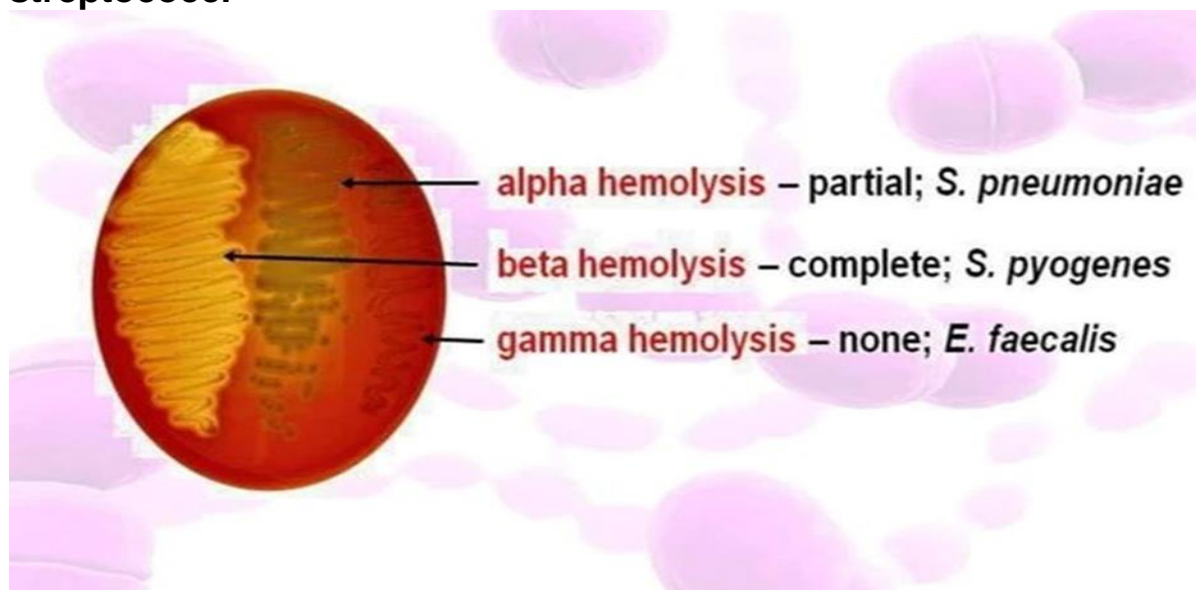


Fig.(1) : Cellular morphology of Streptococcus(Gram positive bacteria)

2. culture(Hemolysis on blood agar)(fig.2,3,4)

Typical haemolytic reactions are produced on blood agar:

- **α-haemolysis:** narrow zone of partial haemolysis and green (viridans) discoloration around the colony, for example, **viridans streptococci**
 - **β-haemolysis:** wide, clear, translucent zone of complete haemolysis around the colony, for example, ***Streptococcus pyogenes***
- **no haemolysis (γ-haemolysis),** for example, non-haemolytic streptococci





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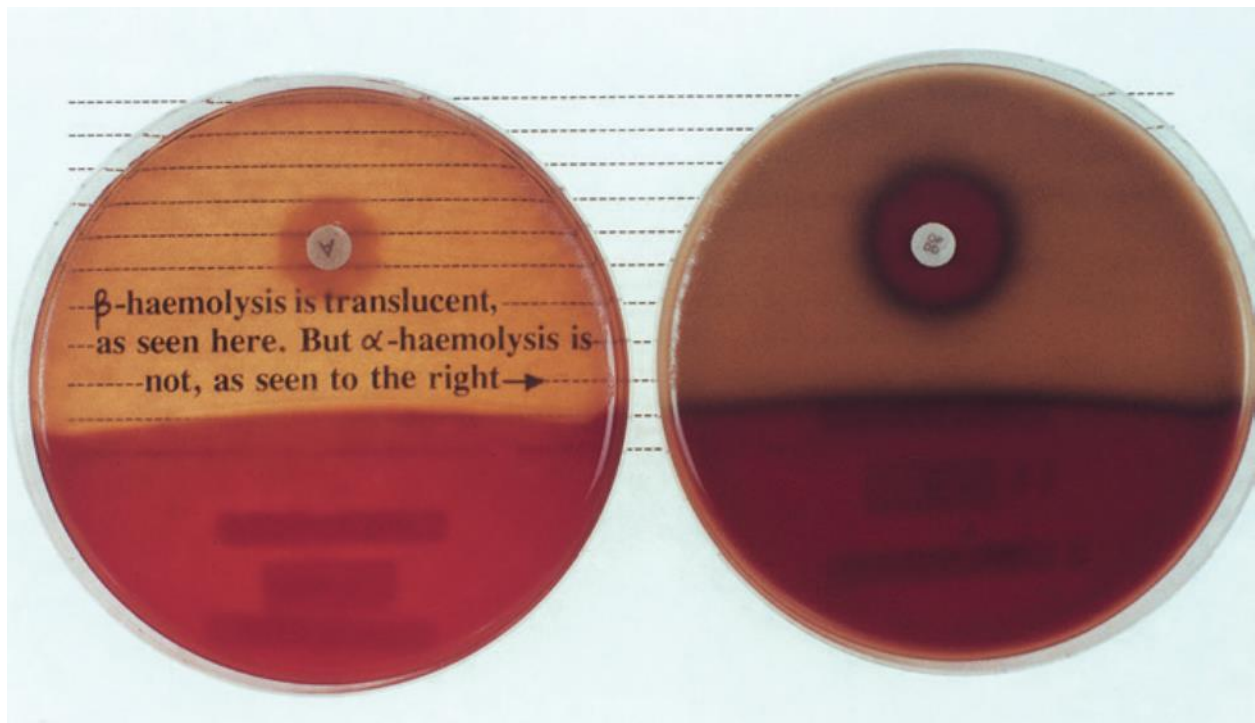


Fig.(2,3,4): α - and β -haemolysis: β -haemolytic colonies (e.g., *Streptococcus pyogenes*) produce complete translucence of blood agar, whereas α -haemolytic colonies (e.g., *Streptococcus pneumoniae*)

3. catalase test (negative results for streptococcus genus) (figure,5)

catalase test

Procedure

1. Label two nutrient broths or nutrient agar slants.
2. Using aseptic technique, inoculate one agar slant or broth with staphylococcus and the other tube with streptococcus.
3. Incubate all the cultures at 37°C for 18 to 24 hours.
4. Add few drops of 3% H₂O₂ over the growth of the slants or broths and observe the appearance of gas bubbles.
5. Record the results.

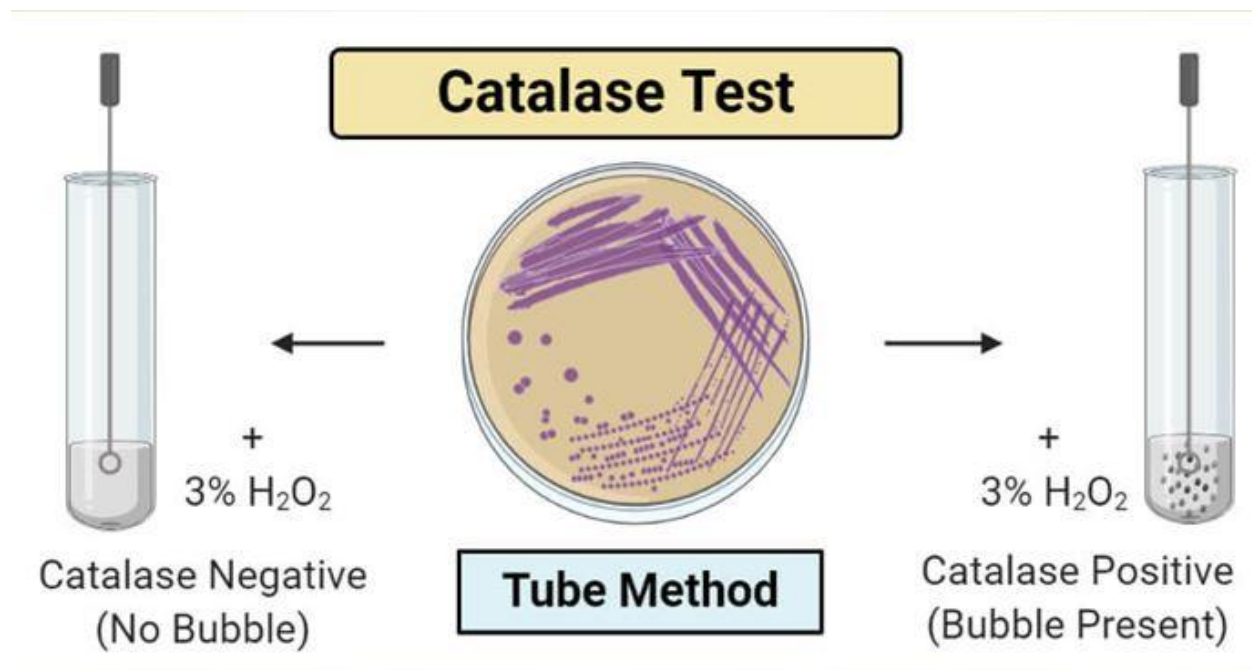


Fig.(5): Catalase Test (tube method)

β hemolytic streptococcus include :

Group A *Streptococcus pyogenes* (group A)

The normal habitat of this species is the human upper respiratory tract and skin. Spread is by airborne droplets and by contact.

It grows well on blood agar, with a characteristic of **β-haemolysis**.

Culture and identification

Culture on blood agar yields characteristic β-haemolytic colonies (**lysis of blood due to streptolysins O and S**).

S. pyogenes, also known as group A Streptococcus (GAS), is the causative agent in a wide range of group A streptococcal infections.

. The most common of these infections include streptococcal pharyngitis (strep throat) , impetigo , scarlet fever . , and bacteremia.

Additional complications may be caused by GAS, the acute glomerulonephritis. acute Rheumatic fever, (a disease that affects the joints, kidneys, and heart valves).

- A Gram-stained smear may show characteristic cocci in chains (Fig. 1);
- The isolate can be presumptively identified as ***Streptococcus pyogenes*** if **it is sensitive to bacitracin**.

Group B

Streptococcus. agalactiae, or group B Streptococcus, GBS, causes pneumonia, meningitis (in neonates and the elderly) and bacteremia..

Differentiation between β-hemolytic streptococci

■ The following tests can be used to differentiate between β-hemolytic streptococci ;

- Lancefield Classification
- **Bacitracin susceptibility Test(fig. 6 and 7)**

Specific test for *Streptococcus. pyogenes* (Group A)

Principle:

- **Bacitracin test is used for presumptive identification of group A**
- To distinguish between *Streptococcus . pyogenes* (**susceptible to Bacitracin**) & non group A such as *S. agalactiae* (**Resistant to Bacitracin**)
- Bacitracin will inhibit the growth of group A Strep. *pyogenes* giving zone of inhibition around the disk

■ **Procedure:**

- Inoculate BAP with heavy suspension of tested organism
- Bacitracin disk (0.04 U) is applied to inoculated BAP
- After incubation, any zone of inhibition around the disk is considered as susceptible

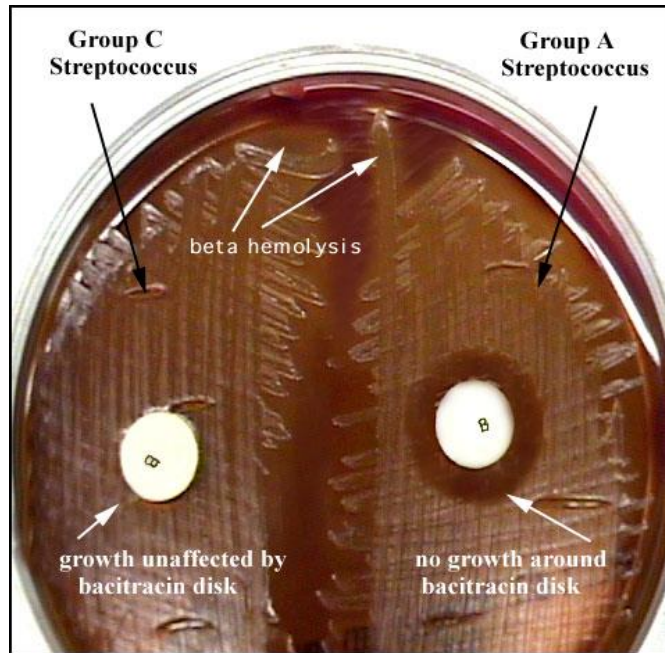


Figure (6): Bacitracin test

Bacitracin sensitivity test

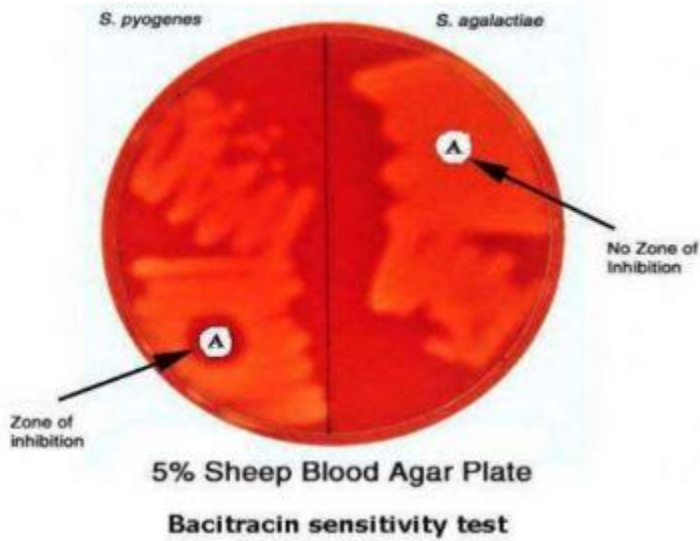


Fig.(7): Bacitracin Sensitivity Test

CAMP test :

- Specific for *Streptococcus. agalactiae* (Group B)

■ Principle:

Group B streptococci produce extracellular protein (CAMP factor)

Alpha-hemolytic streptococcus include :

-This organism causes a number of common diseases, such as pneumonia and meningitis in adults and otitis media and sinusitis in children.

- The viridans streptococci are a large group of commensal bacteria, that are either α -hemolytic, producing a green coloration on blood agar plates , or nonhemolytic that have no Lancefield antigens.
- Including the species *S. sanguinis* , *S. mitis* , *S. mutans* and *S. salivarius*

Streptococcus pneumoniae (pneumococcus)

Characteristics

- Gram-positive 'lancet-shaped' cocci in pairs (diplococci) or short chains; cells are often capsulate;(figure -8)
- α -haemolytic on blood agar;
- catalase-negative; facultative anaerobe .
- Susceptibility to optochin and solubility in bile.
- Observation for the capsular swelling with type-specific antiserum (quellung reaction) confirms the identity and is the standard reference method.

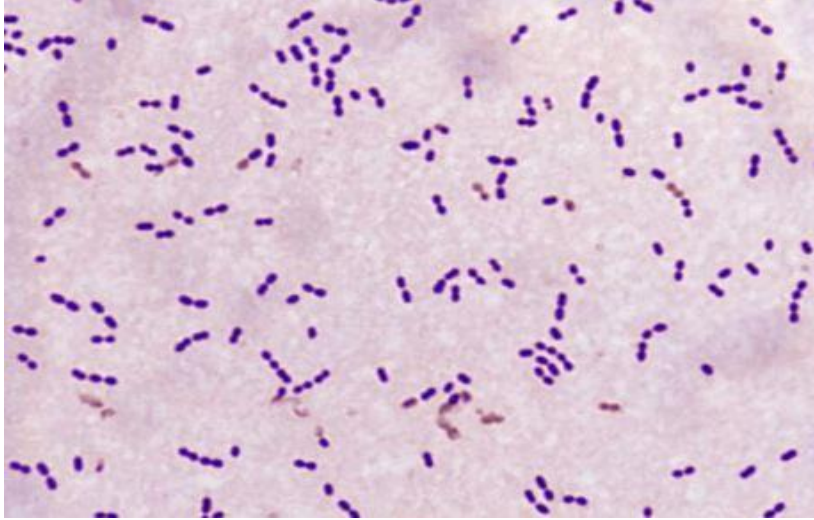


Fig.8 : Streptococcus pneumonia (Gram positive bacteria)

Some biochemical tests must be done on alpha hemolytic streptococci such as the following tests to distinguished between species includes:

A. Optochin sensitivity: (Fig.,9 and 10)

(Optochin Sensitivity Test : positive result Streptococcus pneumoniae , While negative results for Streptococcus viridans).

■ **Principle:**

- Optochin (OP) test that is used to identify Streptococcus pneumoniae
- Strpto.. pneumoniae is inhibited by Optochin.

■ **Procedure:**

- BAP inoculated with organism to be tested
- OP disk is placed on the center of inoculated BAP
- After incubation at 37°C for 18 hours, accurately measure the diameter of the inhibition zone by the ruler
- ≥ 14 mm zone of inhibition around the disk is considered as positive and ≤ 13 mm is considered negative

■ **Results : Streptococcus pneumoniae is positive (Sensitive) while Streptococcus viridans is negative (Resistance)**

A.

+ *S.pneumoniae*, - *S. viridans*.

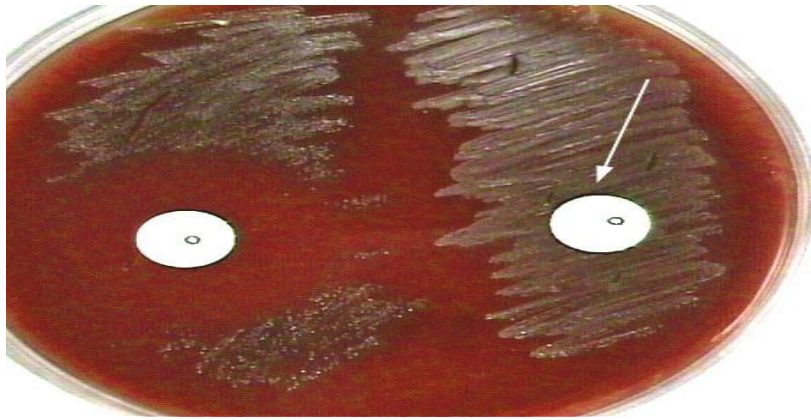
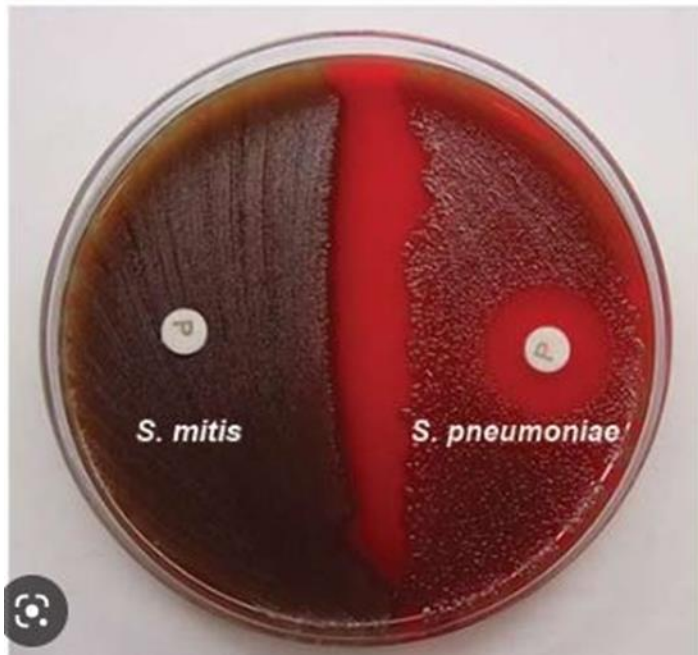


Figure (9) : Optichin sensitivity Test



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

Fig.(10) : Optochin sensitivity test (*s.mitis* resistance to optochin) and *S.pneumonia* susceptible to optochin

B. Bile solubility Test (Figure 11)

positive result for *Strep.pneumoniae*, while negative results for *Strep. viridans*.

■ 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 37oC 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
- *Streptococcus. pneumoniae* soluble in bile whereas *Streptococcus. viridans* insoluble

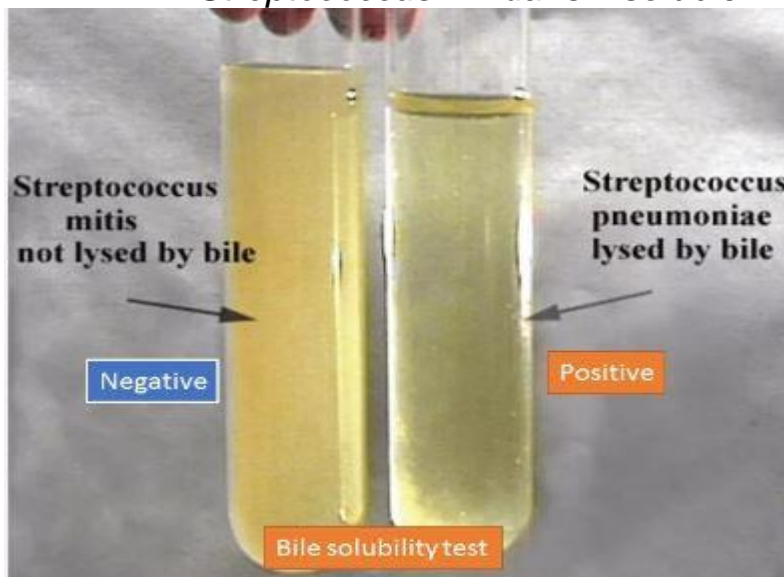


Fig. (11) ; Bile Solubility Test

C. Inuline Fermentation: Positive results for *Strep.pneumoniae* , and negative result for *Strep. viridanse*. (Fig.12)

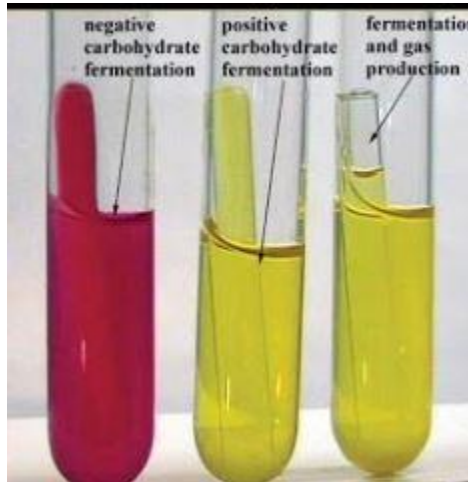
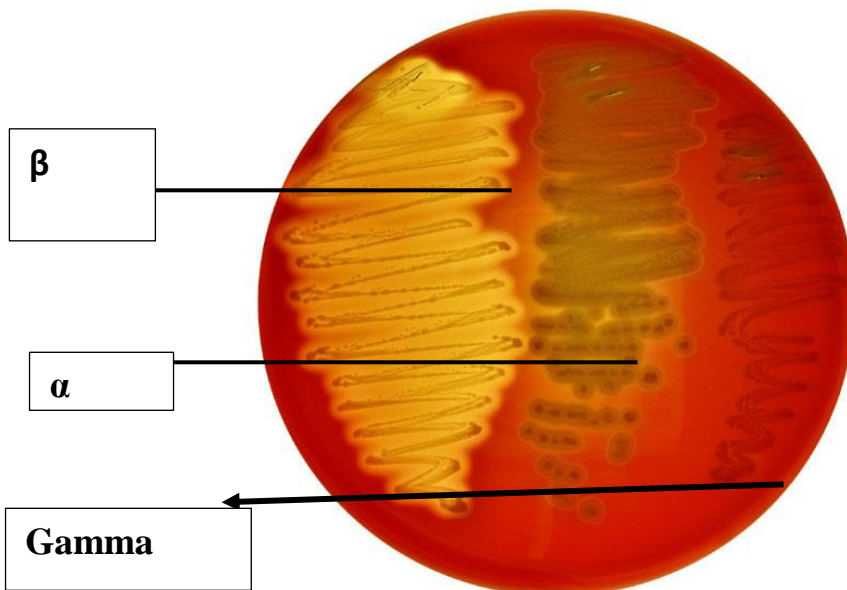


Figure (12) : Inuline Fermentation Test

D. Capsule test : positive result for *Streptococcus. pneumoniae* , and negative result for *Streptococcus. viridanse*.



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Figure (13) : Bacteria on Blood Agar (Hemolysis types)

Procedures for growth of streptococcus

Swab from throat, Gingiva, tongue.

Roll the swab near the edge of a blood agar plate and Mitis Salivarius agar (MSA) (9-10% sucrose) and Manitol Sorbitol Fuchsin agar (MSFA)

Streak with sterile loop, label the plates.

Incubate the plates at 35°C for 24-48 hr.

Choose the small, semi translucent gray-white with sharp border colonies, which surrounded by β -hemolytic zone, this bacteria is considered as *Strep. pyogenes*.

Choose other colonies, that are small surrounded by greenish-brown zone, and prepare slides for gram-stain which is considered as *Strept. viridians* .

From non-hemolytic colonies should be found the other, species that could be identified by biochemical, and immune test.

Examine the colony morphology of streptococci on MSA (Figure 14), which are large, blue in color, raised and mucous due to the formation of extracellular capsule. On MSFA colonies are pink to red according to the bacterial species.



Figure (14) : Mitis salivarius agar and oral streptococci

The End

