Genera Streptococcus & Enterococcus

Dr. Ahmed Hussein Abed

Assistant Prof. of Bacteriology, Mycology and Immunology

General Characteristics of G. Streptococcus

- In 1984, many organisms formerly considered Streptococcus were separated out into the genera Enterococcus and Lactococcus.
- Currently, over 50 species are recognized in this genus.
- They are **Gram-positive**, ovoid cocci arranged mostly in pairs or **chains** (long in fluid media or short on solid media).
- They are the smallest cocci (0.5- 0.8μ in diameter).
- They are non-motile, non-sporulating. The pathogenic Streptococci are capsulated (e.g. *S. pneumoniae* have thick capsules of Hyaluronic acid and produce mucoid colonies)

- Many streptococci are present as normal flora in milk and its products, buccal cavity, GIT and respiratory tract of man and animals.
- Some species are highly pathogenic for man and animals.
- Most of pathogenic streptococci have carbohydrate antigen.
- **Catalase & Oxidase negative**: Rapidly differentiates from *Staphylococci.*
- Aerobic or facultative anaerobic.
- They are **fastidious** bacteria and require the addition of **blood**, **serum** or **glucose** to culture media.

Genus Peptostreptococcus: Anaerobic Streptococci e.g. *Peptococcus indolicus* which is the aetiology in association with *Arcanobacterium pyogenes* of bovine summer mastitis.

G. Enterococcus

- Enteric Streptococci found in the intestinal tract of animals and man.
- They are larger than Streptococci in size (0.8-1.2μ in diameter) e.g. *E. fecalis*.
- They are opportunistic pathogens.
- They are differ from the Streptococcus species in two important respects:

✓ They tolerate NaCl 6.5% as well as bile salts; so they can grow on MacConkey agar as red, pin-point colonies.

✓ Some isolates are motile.

N.B. Recently, Some members are catalase positive.

❑ Enterococci have emerged as pathogens of human and several domestic species, causing enteritis, septicaemia, mastitis, respiratory diseases and urinary tract infections.

Classifications of Streptococci

I. Brown's classification: (Type of haemolysis on sheep or ox blood agar)

A. Beta "β" haemolytic

- Complete haemolysis indicated by clear zones around colonies.
- Most of pathogenic streptococci belong to this group.
- e.g. *S. pyogenes, S. equi and S. agalactiae.*

B. Alpha "α" haemolytic

- Partial or incomplete haemolysis indicated by greenish or hazy zones around colonies.
- It includes Viridans group (*S. viridans*) and *S. pneumoniae*.

C. Gamma "γ" haemolytic (Non haemolytic)

- Denotes no observable changes in the blood agar around colonies.
- e.g. *Enterococcus faecalis*.

Classifications of Streptococci

II. Lancefield grouping: It is a serological method of classification based on the group-specific C-substance (carbohydrate or polysaccharides Ag) in the cell wall using Ring precipitation test OR Latex agglutination test.

- ✓ They include 20 serogroups with sequentially letters **A-H** and **K-V**.
- ✓ Some Streptococci are **non-groupable** such as **Viridans** group and ***S. pneumoniae***.
- ✓ Groups **A, B, C, D** and **G** are the most common human and animal pathogens.
- ✓ Groups A, B, C and G are **β-hemolytic** while D and other groups or non-groupable Streptococci are mostly α or γ hemolytic.

Pathogenic Streptococci, their habitats, hosts and consequences of infection.

Species	Lancefield group	Haemolysis on blood agar	Hosts	Consequences of infection
S. pyogenes	A (polysaccharide)	β	Man	Scarlet fever, septic sore throat and rheumatic fever
S. agalactiae	B (polysaccharide)	β (α, γ)	Cattle, sheep, goats, man, dogs	Chronic mastitis and neonatal septicaemia
S. dysgalactiae	C (polysaccharide)	β (α, γ)	Cattle	Acute mastitis
S. equisimilis (S. dysgalactiae subsp. Equisimilis)	C (polysaccharide)	β	- Horses - Cattle, dog and birds	- Abscesses, endometritis and mastitis - Suppurative conditions
S. equi	C (polysaccharide)	β	Horses	Strangles, suppurative conditions and purpura haemorrhagica.
S. zooepidemicus	C (polysaccharide)	β	- Horses - Cattle, lambs, poultry	- Mastitis, pneumonia and navel infection - Suppurative conditions and septicaemia
E. faecalis	D (Teichoic A)	γ	Many species	Suppurative conditions following opportunistic invasions
S. ubris	Not assigned	α (γ)	Cattle	Mastitis.
S. pneumonia	Not assigned	α	Man, primates, G. pigs, rats	Septicemia, pneumonia and meningitis.

Pathogenicity: virulence factors

- Pyogenic Streptococci are associated with abscess formation, other suppurative conditions and septicaemias.
- Beta-hemolytic Streptococci are generally more pathogenic than those producing alpha-haemolysis.
- Virulence factors include enzymes and exotoxins & others.

❑ **M protein: (Cell wall protein)**

✓ Streptococcal virulence is based in large part on antiphagocytic surface components, including the M protein

✓ M protein is one of two major protein classes (M& T antigens). There are also 2 minor classes (R& F) but M protein is considered the only virulence factor among them.

✓ It is type-specific antigen, Fimbriae-like (adhesion).

✓ Resistant to heat and acid but trypsin sensitive.

❑ **Capsule:**(antiphagocytic) Not present in all strains.

✓ It may be polysaccharide (*S. pyogenes*) or hyaluronic acid (*S. pneumoniae*).

❑ Pyrogenic exotoxin A:

✓ It is one of 9 super antigens that contribute to the pathogenesis of streptococcal toxic shock syndrome by stimulating cytokine production by T-cells, with subsequent endothelial cell damage, hypotensive shock, and ischemia-based necrosis.

❑ Streptokinase (Fibrinolysin):

✓ It dissolves fibrin clots (spreading factor). It is produced mainly by Group A and some members of groups C& G.

❑ Hyaluronidase:

✓ Spreading factor by destroying hyaluronic acid of host tissues. It is produced mainly by groups A and B.

❑ DPNase:

✓ Toxin similar to leukocidin of *S. aureus* destroying WBCs resulting in pus formation.

❑ Erythrogenic toxin:

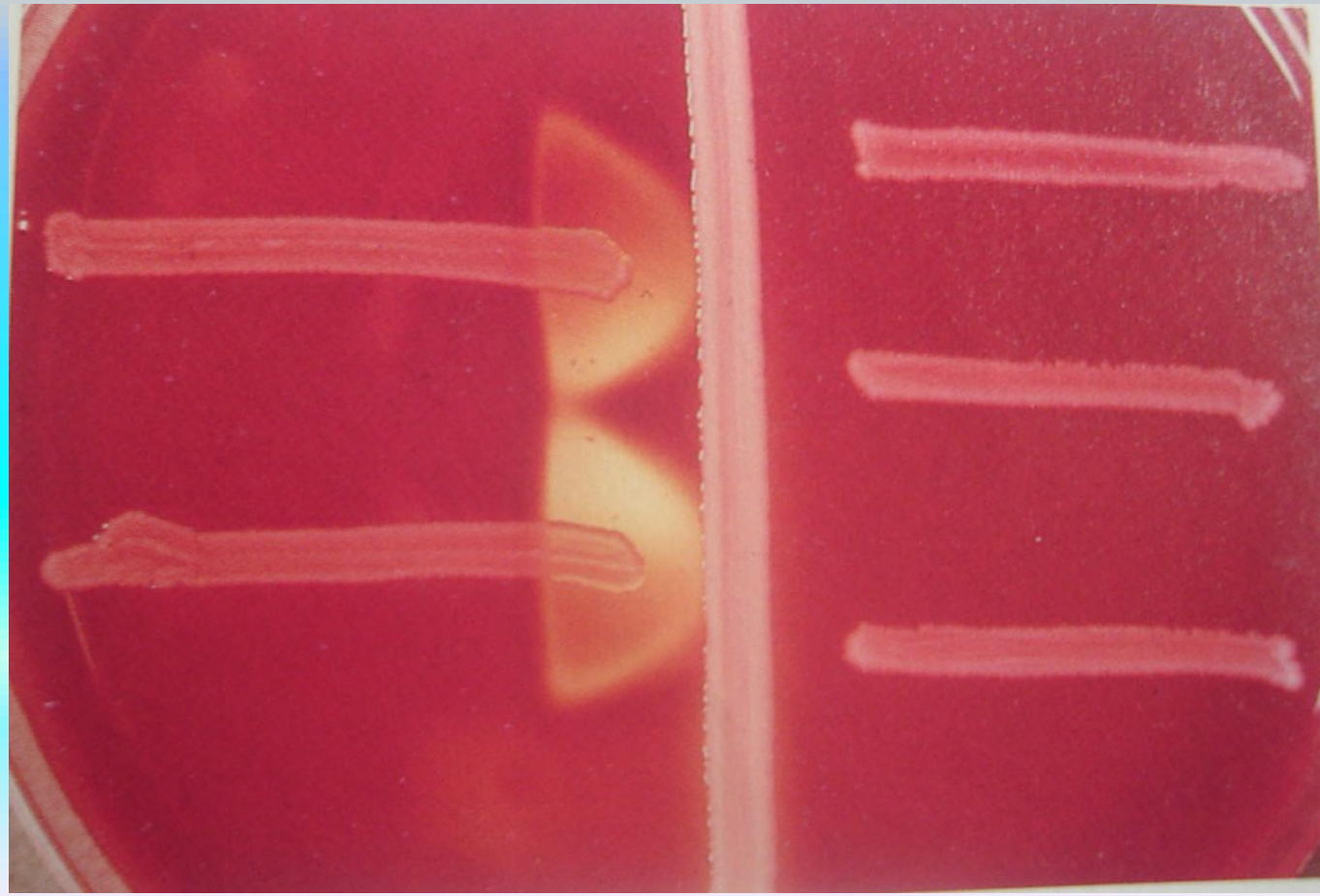
✓ It is produced by group A especially *S. scarletina* causing skin redness (scarlet fever).

- ❑ DNase, NADase and proteases: These enzymes also are contribute to virulence.
- ❑ **Streptolysin O (SLO): Oxygen-labile haemolysin**
- ✓ It is a cholesterol binding toxin with potent membrane damaging effects.
- ✓ It is produced by groups A, C, and G and act under reduced conditions.
- ✓ It is highly antigenic; stimulating antistreptolysin O (ASO) production in case of sub acute& chronic *S. pyogenes* infections.
- ✓ High titer of ASO (more than 166-200 units) indicates active infection which could be complicated by rheumatic fever or rheumatic heart.
- ❑ **Streptolysin S: Oxygen-stable haemolysin**
- ✓ It is responsible for haemolysis on blood agar plates.
- ✓ It is produced by haemolytic strains.
- ✓ It is not antigenic.

❑ CAMP factor:

- ✓ *S. agalactiae*, the only member of group B, is best known as a cause of chronic bovine mastitis.
- ✓ Strains of *S. agalactiae* augment the hemolytic activity of Staphylococcus β-toxin via the action of CAMP factor.
- ✓ CAMP factor is lethal for laboratory animals (rodents).

CAMP test for confirmation of *S. agalactiae*, vertical streak: *S. aureus*, left horizontal inoculation streaks: *S. agalactiae* with cup-shaped (head of an arrow) haemolysis, 48 hrs, 37C.

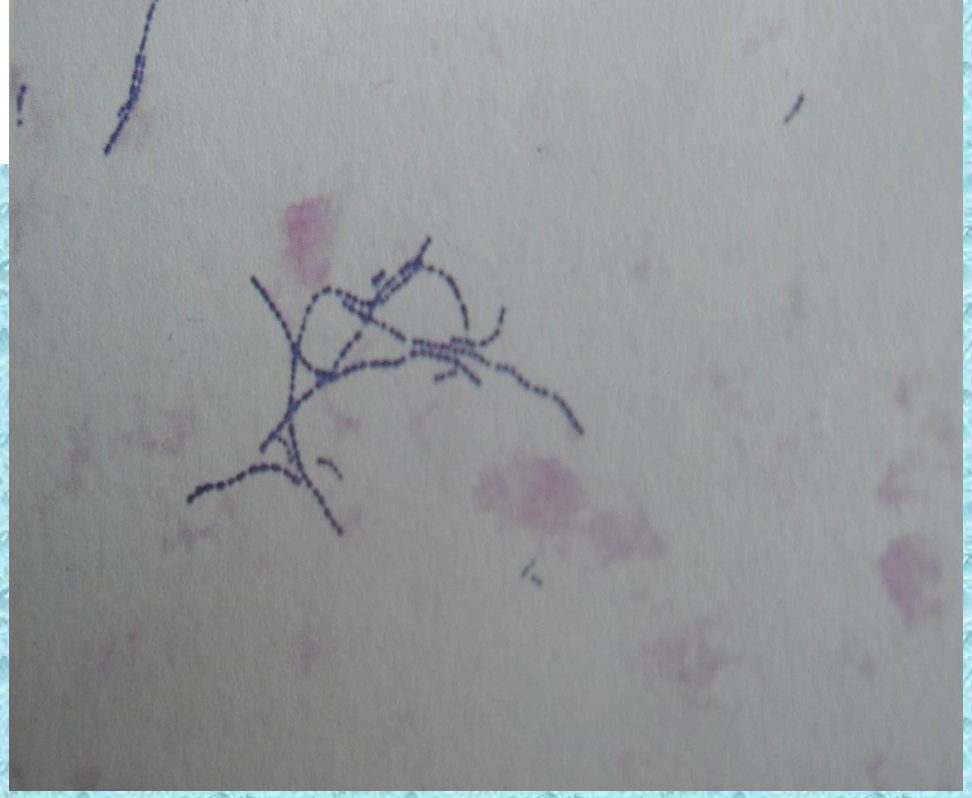


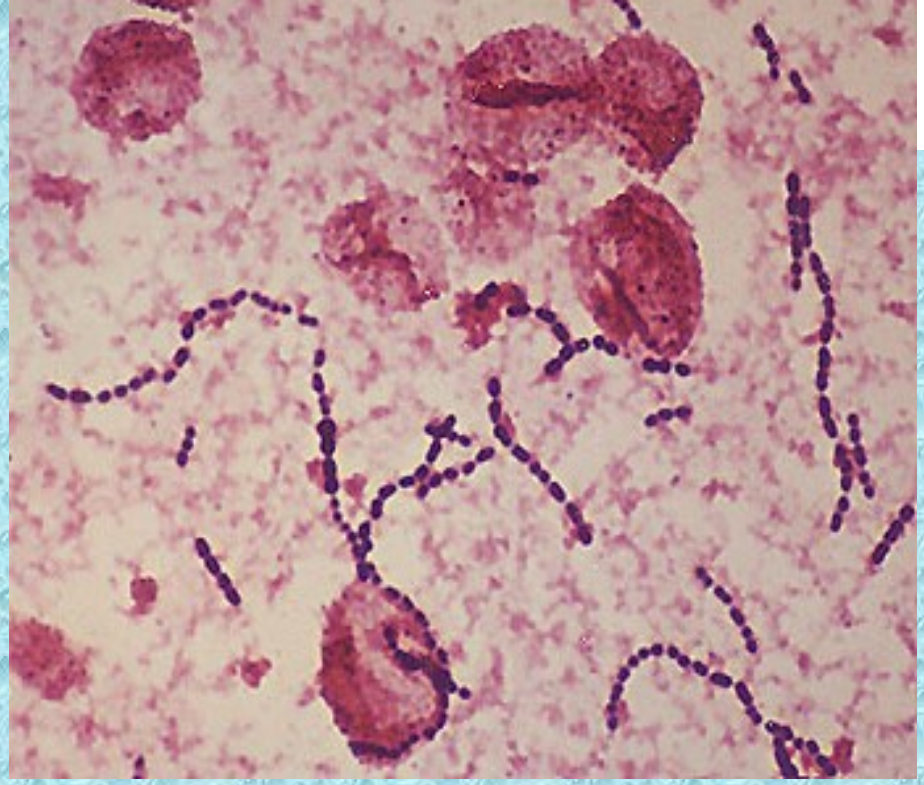
Laboratory Diagnosis

- Specimen: According to hosts, habitats and disease e.g milk (mastitis), throat swab (strangles, tonsillitis, septic sore throat), pus (abscess),.....etc.
- N.B. In case of human and horse only, indirect diagnosis (serodiagnosis) is done by using collected serum samples to titrate ASO.

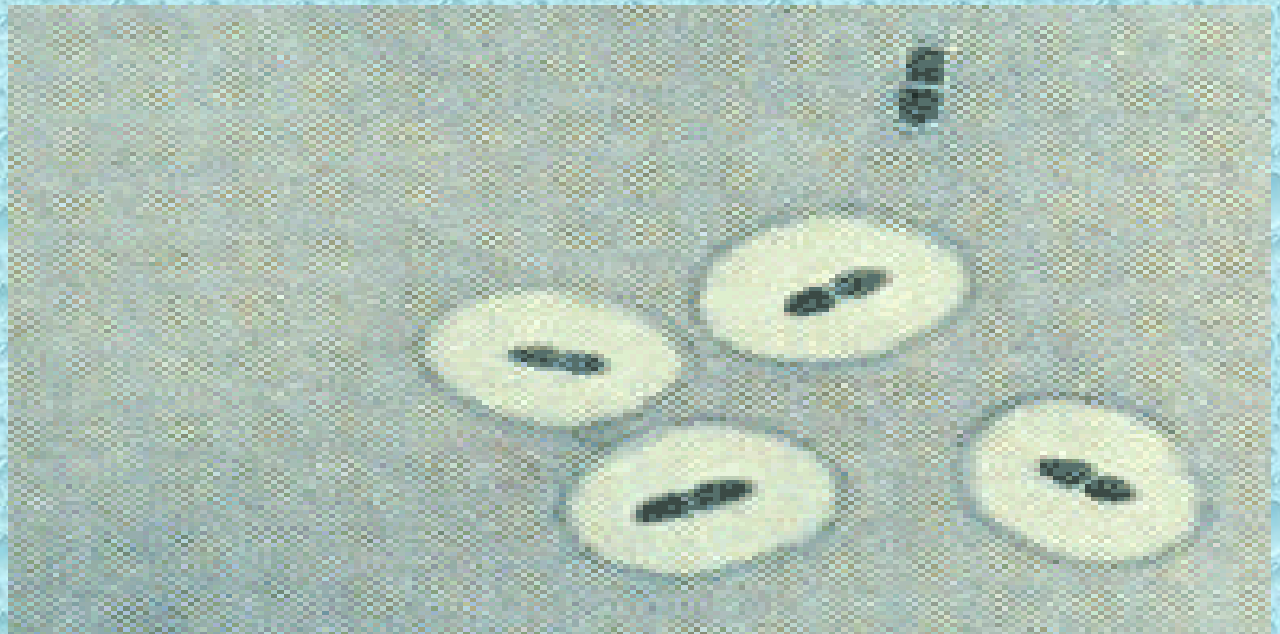
Morphology

- It is detected by microscopical examination of direct film from samples or culture stained by Gram`s stain to detect typical morphology. In case of *S. pneumonia*, diplococci are seen lancet shape with thick capsule.
- Loeffler's Methylene Blue is used in case of *S. agalactiae* in mastitic milk. In positive cases; long chains of Streptococci are distributed between the fat vacuoles.





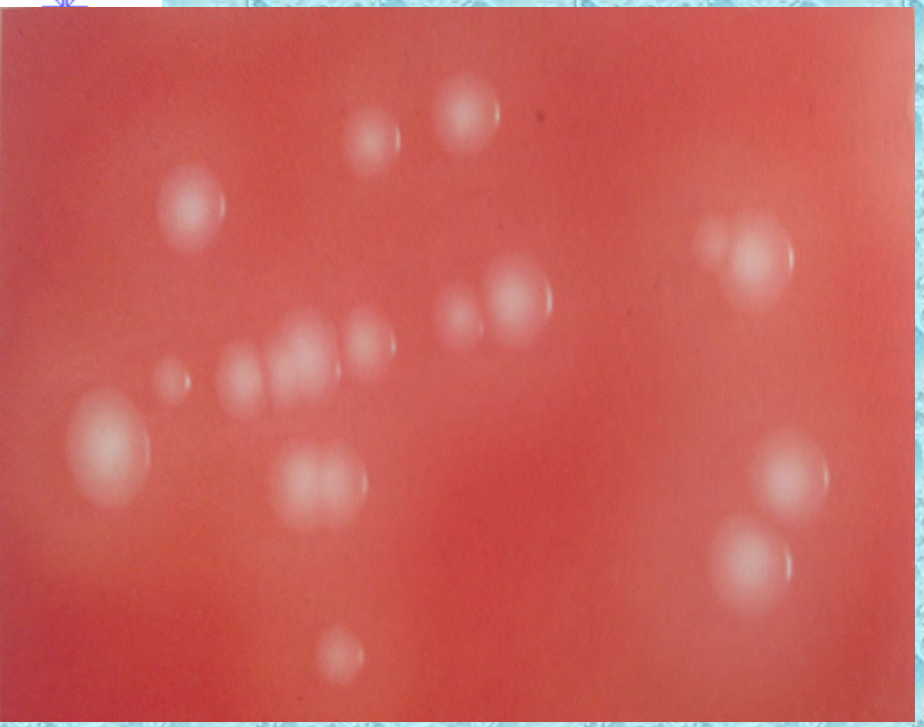
Diplococci lancet shape with thick capsule



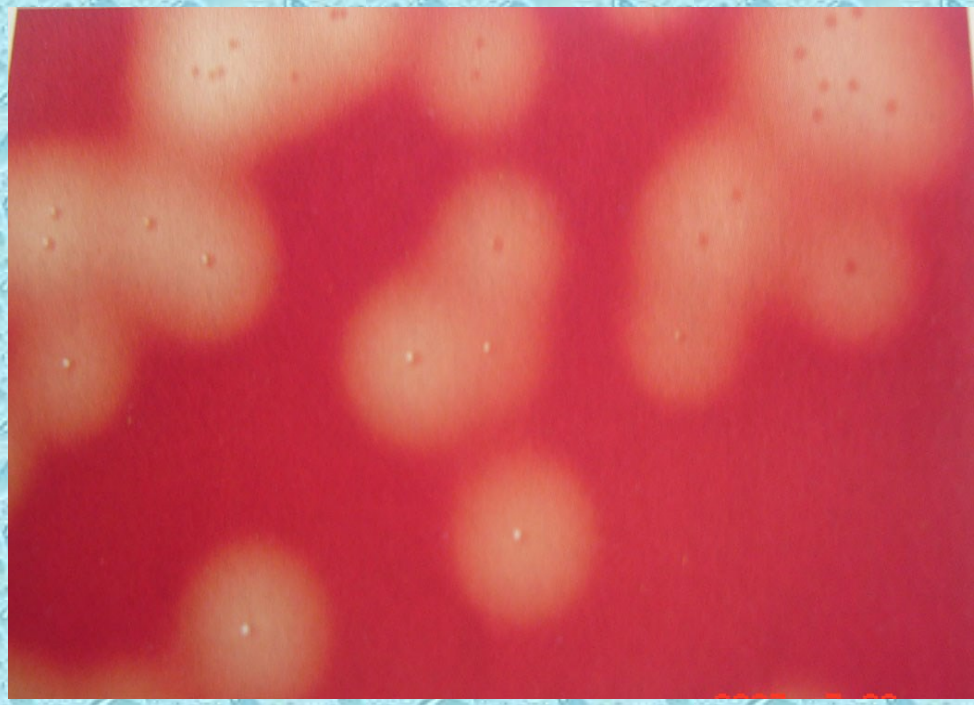
Culture charcters

- Aerobic and facultative anaerobic
- Fastidious bacteria require enriched media (supplied with blood, serum or glucose).

- **Brain heart infusion or serum agar:** Colonies are small; **dew like drops** or **pin-point**, translucent, some of which may be mucoid (capsule).
- **Glucose broth cultures:** colonies are faint granular growth, powdery sediment with clear supernatant.
- **Blood agar or Selective blood agar:** to detect type of haemolysis.
- **MacConkey agar:** ***Enterococci*** grow giving as red, pin-point colonies.



***S. agalactiae*, pure culture on blood agar, weak haemolysis, 48 h, 37˚C.**



***S. zooepidemicus*, pure culture (pin point clonies) with β-haemolysis on blood agar, 24 h, 37˚C.**

Biochemical reaction

- **Catalase** and **Oxidase** –ve.
- Fermentation of glucose and maltose with production of acid only.
- Reduction of **Litmus milk** and **Methylene blue** is specific for ***Enterococci***.
- **Bile solubility**: bile 40%: ***S. pneumonia*** is the only strain causing bile solubility meanwhile; ***Enterococci*** can grow only in bile.
- **CAMP** Test: for identification of ***S. agalactiae***.

Serological tests

- **Lancefield grouping:** To detect C-antigen by using:

✓ Ring precipitation test: The C-substance is extracted by acid or heat. This antigen extract is layered over antisera of different specificities in narrow tubes. A positive reaction is indicated by the formation of a white ring of precipitate close to the interface of the two fluids within 30 min.

✓ Latex agglutination test: Specific C-substance antisera for groups A to G are commercially available. Suspensions of latex particles are coated with each of the group-specific antibodies. A drop of antigen is mixed on a plate with a drop of each latex-antibody suspension and rocked gently. A positive reaction is indicated by agglutination within one minute.

- In case of human and horse only, agglutination tests are used to detect and titrate ASO.

Molecular techniques

- **PCR:** A sensitive technique has been developed for detecting ***S. equi*** in nasal swabs.