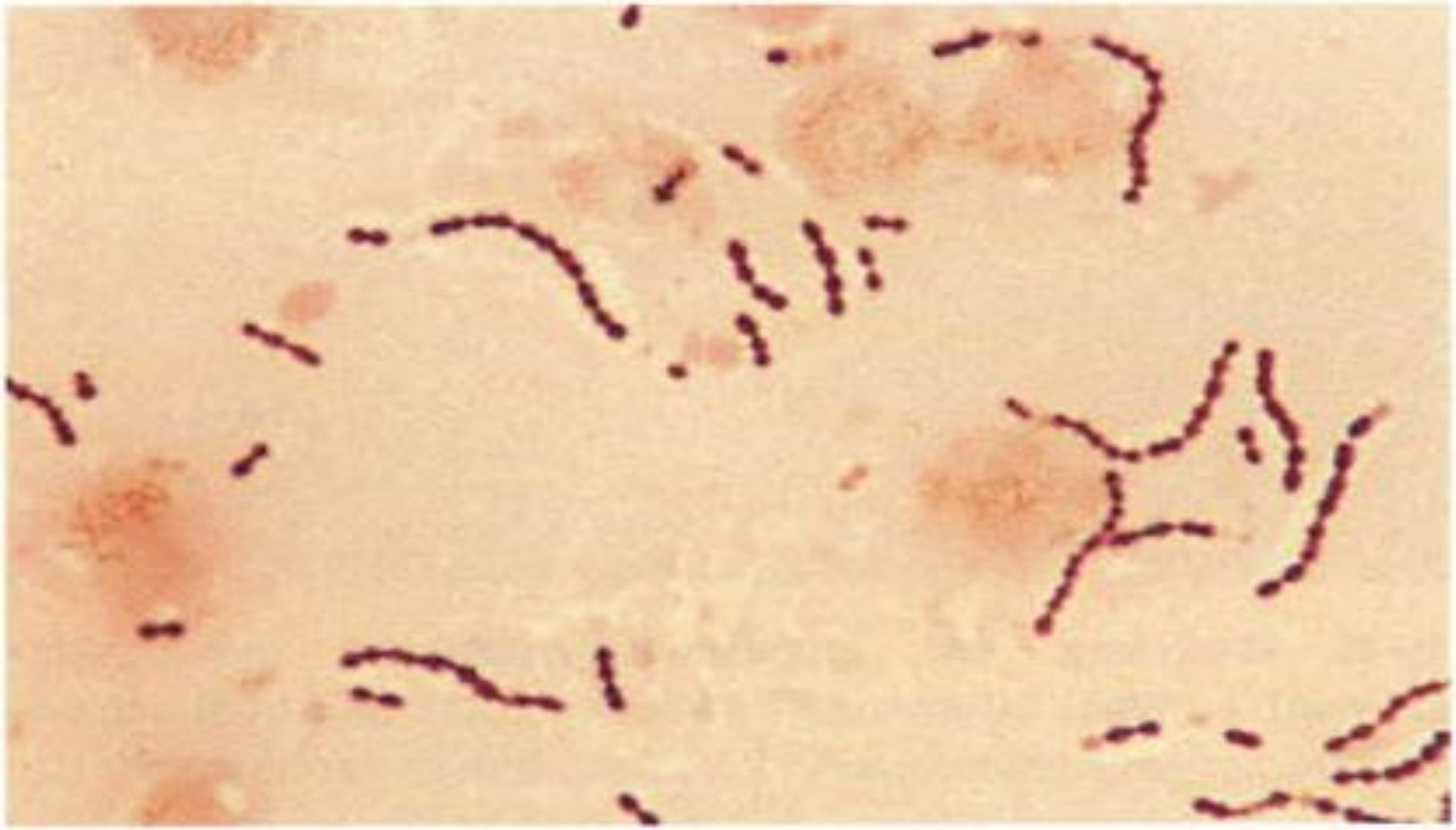
Streptococci

Streptococci are Gram positive, non motile, non spore forming catalase negative cocci that occur in pairs or chains, older cultures may lose their Gram-positive character

Most Streptococci are facultative an aerobes, and some are obligate (strict) an aerobes.



6-7 Gram stain of streptococci in broth culture (×1250). Gram stain of a positive blood culture broth demonstrating gram-positive cocci arranged in chains. Streptococci are normal microbiota in the upper respiratory tract and the gastrointestinal tract. For this reason, Gram stains of specimens from these sites are not helpful in diagnosing infections caused by the pathogenic streptococci, such as *S. pyogenes* and *S. pneumoniae*.

Classification Based on Lancefield Proteins

Rebecca Lancefield, working with various streptococcal species, discovered proteins in the cell wall that were unique to certain organisms. These proteins were labeled Group A, Group B, Group C, and so on through Group M. Currently three Lancefield Groups are of medical importance: Group A, Group B, and Group D.

- Of the organisms used in this lab the following correlations apply:
- Group A Strep--*Streptococcus pyogenes*
- Group B Strep--*Streptococcus agalactiae*
- Group D Strep--*Streptococcus bovis, Enterococcus (Streptococcus) faecalis*
- *Streptococcus pneumoniae* does not possess Lancefield proteins and is not classified in one of the Lancefield groups. *Viridans streptococci* is the term applied to alpha- hemolytic Streptococcus species that lack Lancefield proteins

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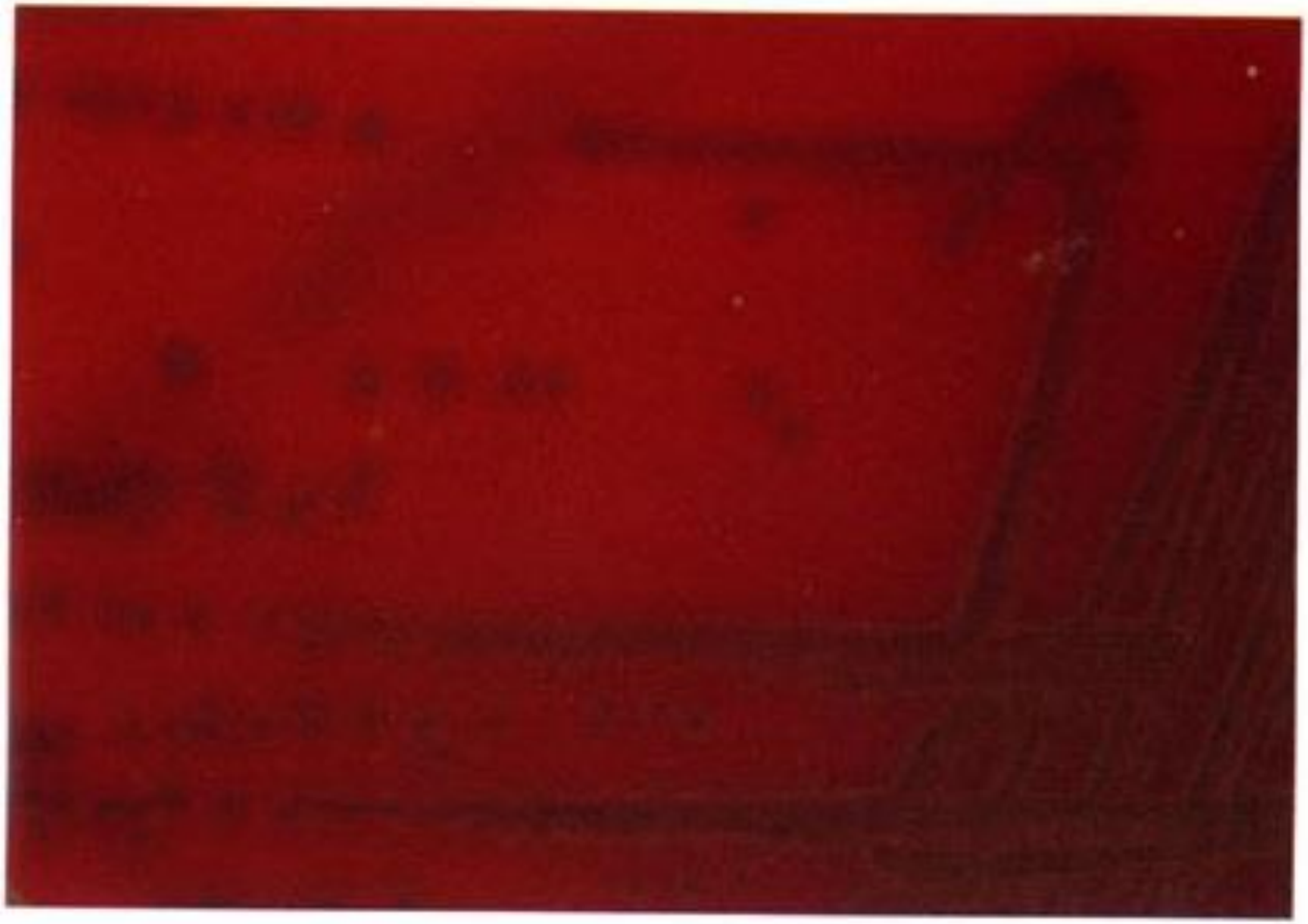
Specimens

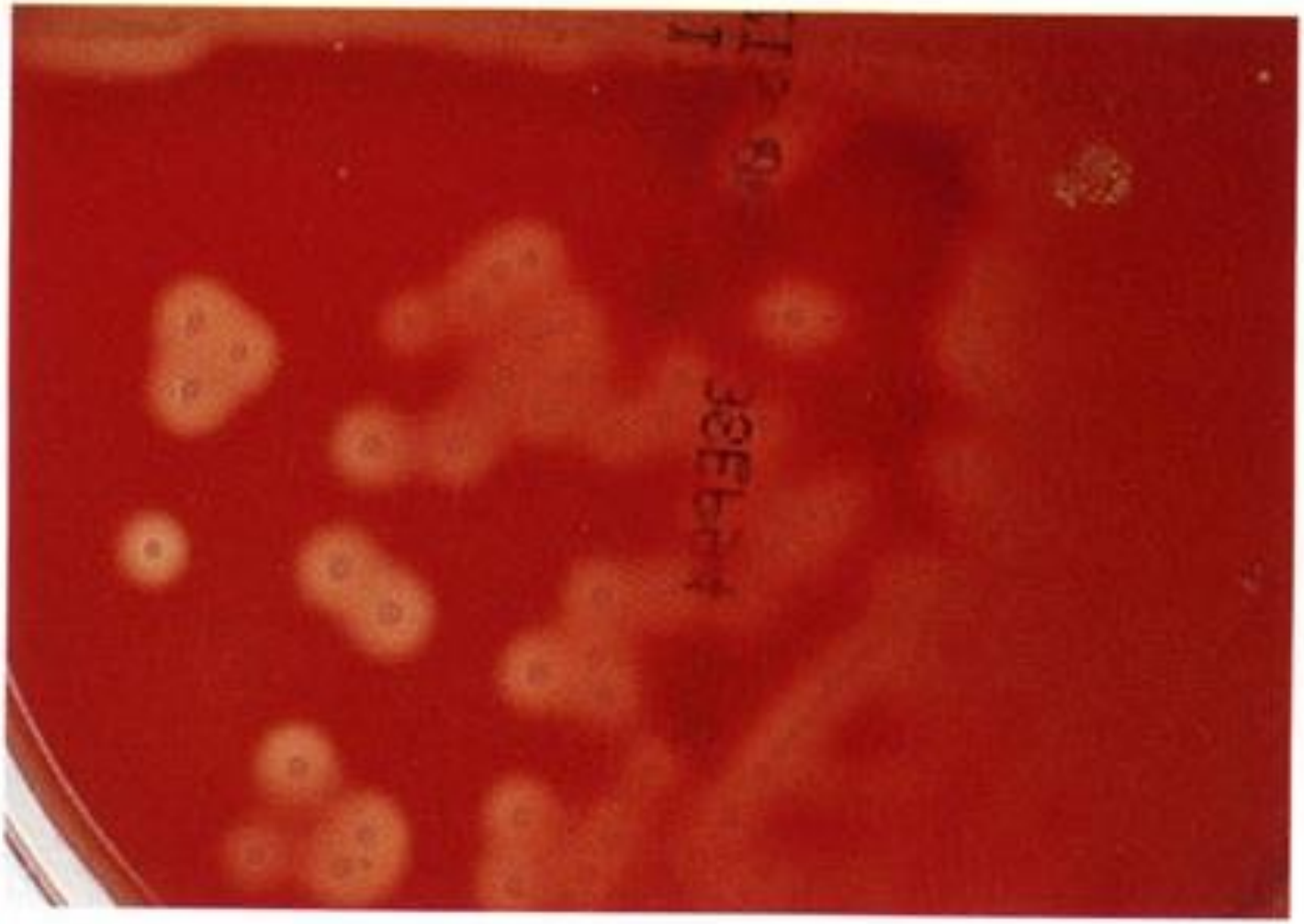
- Throat swab, pus, sputum, blood, urine, vaginal swab, feaces, peritoneal fluid, gingival swab, carious lesion swab .

Lab diagnosis

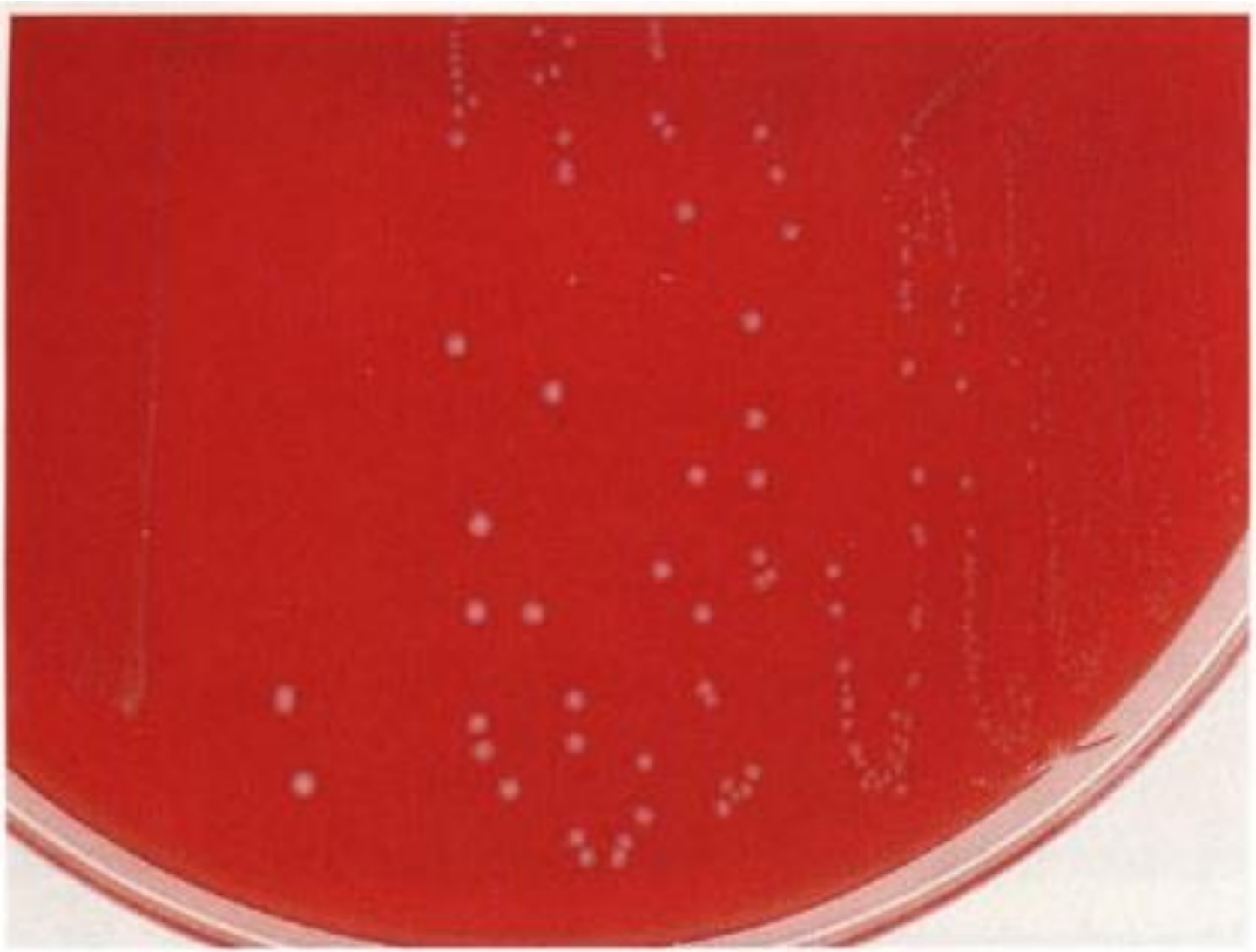
- Gram stain
- Colonial Morphology on blood agar.
- Catalase test.
- Blood hemolysis
- Optochin disc sensitivity test.
- Bile solubility test.
- Capsular swelling.
- Growth 6.5%NaCl
- Bacitracin disc test.
- CAMP test



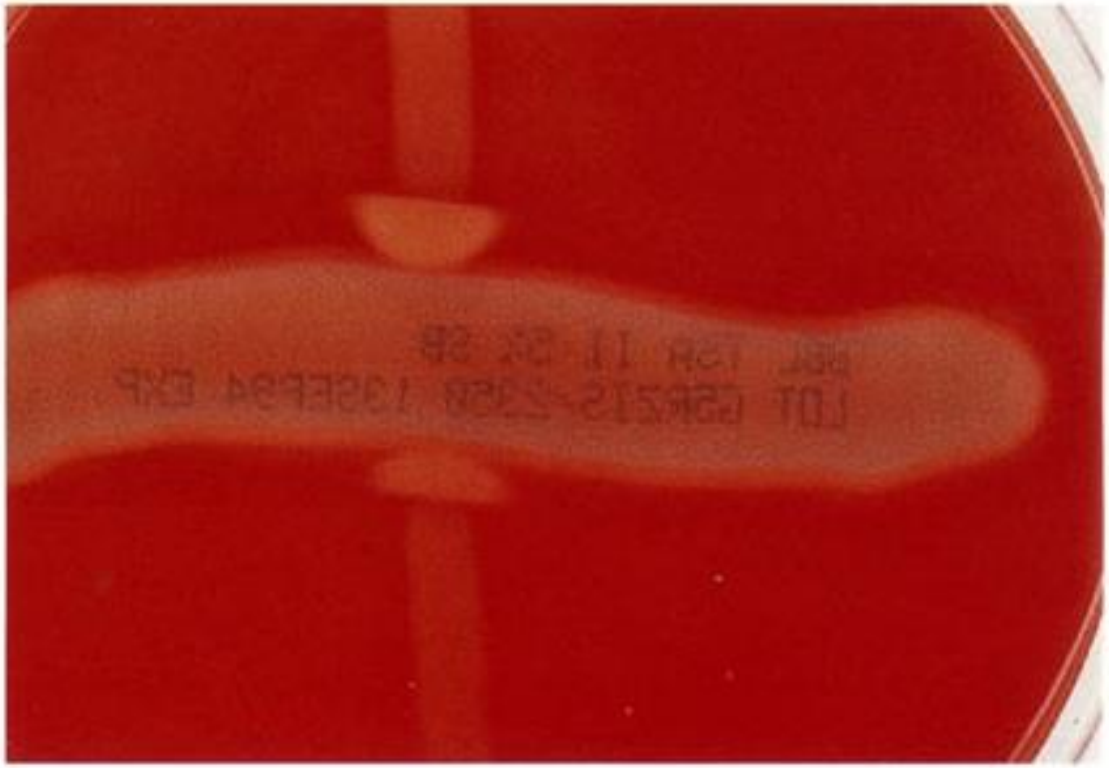
6-1 Alpha hemolysis on 5% sheep blood agar plate. Alpha hemolysis is an indistinct zone of partial lysis of red blood cells (RBC) causing a green to greenish-brown discoloration of the medium immediately surrounding the colony. Viridans streptococcal colonies and pneumococcal colonies are alphahemolytic.



6-3 Beta hemolysis on 5% sheep blood agar plate. With beta hemolysis, there is complete lysis of RBC surrounding the colony that can readily be seen macroscopically.



6-5 Gamma hemolysis on 5% sheep blood agar. *Gamma* is a term used to denote lack of hemolysis; the RBC surrounding the colonies are intact.



6-15 CAMP Test. A positive CAMP test for group B streptococci demonstrating the arrowhead-shaped enhancement of beta hemolysis that occurs when the hemolytic beta-toxin produced by *S. aureus* (the microorganism streaked horizontally across the sheep blood agar plate in this photograph) acts synergistically with the CAMP factor protein produced by Group B streptococci (streaked perpendicular to the staphylococcus but not quite touching). The CAMP test, which is named for its discoverers, Christie, Atkins, and Munch-Peterson, is an alternative to hippurate hydrolysis.

Toxins and enzymes of Strepcocci

- Streptokinase(fibrinolysin): an active proteolytic enzyme that digests fibrin and other proteins . It is produced by many strains of group A β-hemolytic streptococci.
- Hemolysin : streptococci produce 3 kinds of hemolysin
- Streptodornase (streptococcal deoxyribonuclease) depolymerizes DNA.
- Hyaluronidase (spreading factor): splits hyaluronic acid, an important component of connective tissue(digest host connective tissue) .

- Streptolysin:
- 1-Streptolysin (S): is an oxygen – stable cytolysin.
- 2-Streptolysin (O) is reversibly oxygen – labile cytolysin. Anti-Streptolysin O test (ASO test) or ASO titer.
- Protease: causing soft tissue, necrosis, toxic shock syndrome
- Streptococci are classified on the basis of colony Morphology, hemolytic, biochemical reactions and serologic specificity. They are divided into three groups by the type of hemolytic on blood agar:

- **β** - hemolytic (clear, complete lyses of red cells): *s. Pyogens, s. agalagtiae.*
- ∞- hemolytic (incomplete, green color):*s. pumoniae, viridans streptococci*
- γ-hemolyticc (no hemolytic). *s.foecalis.*
- Serologic grouping is based on antigenic differences in cell wall carbohydrates (group A to V) in cell wall pilli associated protein, and in the polysaccharide capsule .

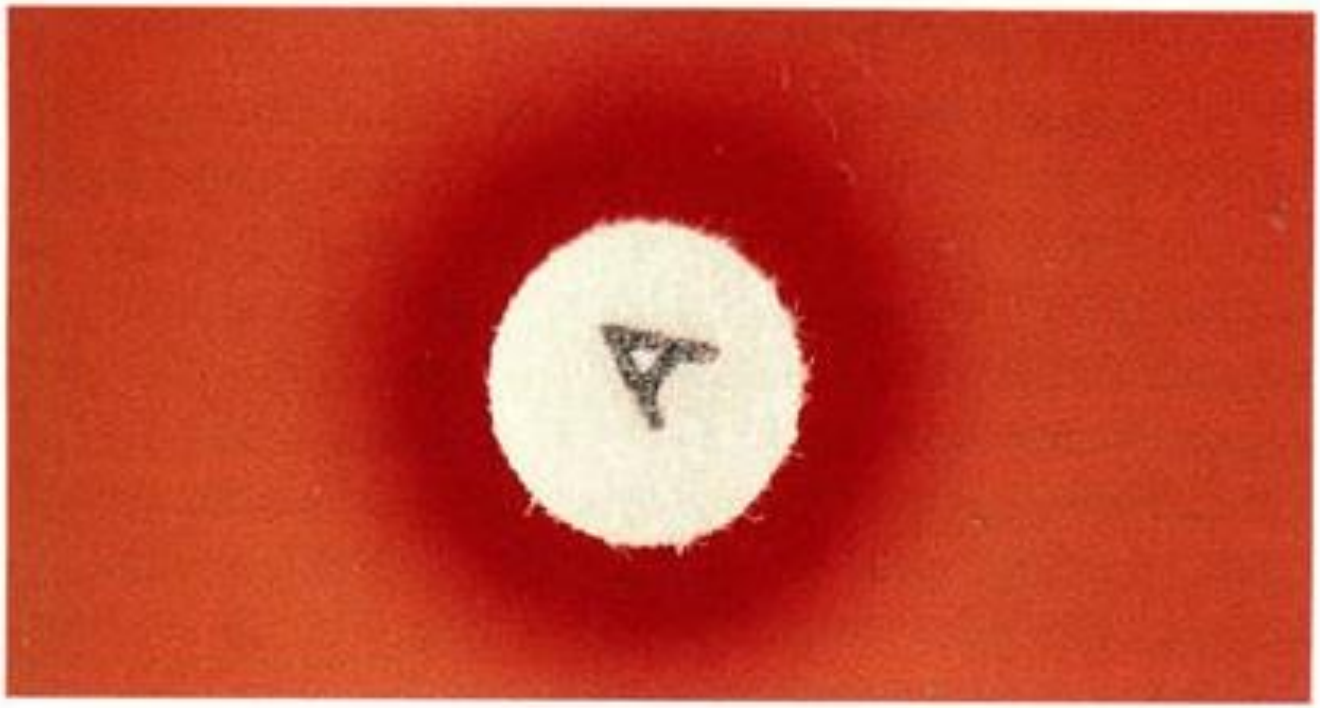
Bacitracin susceptibility

Procedures

1. Divide a sheep blood agar plate into four quadrants.
2. Label a quadrant with the name of the organism to be tested.
3. Using a sterile loop aseptically transfer the test organism to the plate and streak the quadrant for confluent growth

4. Aseptically transfer a bacitracin disc (A disc) to the center of the quadrant. Forceps may be used to position the disc. Gently press the disc to the surface of the agar but do not embed the disc in the agar.
5. Invert the plate and place in the incubator for a minimum of 18 hours.
6. Examine the plate for a zone of inhibition of growth around the disc. When finished discard the plate in the biohazard container.

Interpretation: Any zone of inhibition of growth is considered positive (+) for this test. If a red ring can be seen around the disc this is considered a positive test (*S. pyogenes)*.



6-11 Bacitracin susceptibility test. The bacitracin susceptibility test is an alternative to the PYR test for the presumptive identification of group A beta-hemolytic streptococci. A 0.04-U bacitracin disk is placed on an inoculum of the microorganism on sheep blood agar. After overnight incubation at 35°C, any zone of inhibition is interpreted as a positive test, and the microorganism is presumptively identified as group A streptococci by bacitracin.

Table (1) :Medically Important *Streptococci*

Type species	Lance field serogroup	Normal habitat	Significant human disease
S.pyogenes	A	Human	Acute pharyngitis,A cute glumerulonephritis, Scarlet fever, Rheumatic, fever. Cellulites
S.agalactiae	B	Cattle, humans	Neonatal meningitis and sepsis and infections.
S.equisimitis S.faecalis	C	Wide human and animal distribution	Endocarditis, bacteremia, pneumonia, upper respiratory infection.
S.bovis (non enterococcu)	D	Human and animal intestinal tract,	Billiary or urinary tract infection, endocarditic.
S.anginosus	F,G	dairy products bacteremia	Subcutaneous or organ abscesses endocarditic, upper respiratory infection.
S.sanguis	H	Human, animals	Endocarditic, caries.
S.salivarius	K	Humans	Endocarditic, caries.
Non	O	Humans	Endocarditic.
S.suis	R	Humans	Meningitis.
"*S.Viridians*" *S.miti S.mutans*	Non identified	Humans	Caries, endocarditic
Anaerobic or microaerophilic	Non identified	Wide human and animal distribution	Brain and pulmonary abscesses gynecologic
S.pneumoniae	Non identified	Humans	Lobar pneumonia and others

- **<u>Optochin Susceptibility:</u>**
- This test was done as same as the bacitracin test(used optochin disc).
- Interpretation: A growth inhibition zone of 15-30 mm is considered a positive (+) test(*S. pnumoniae)*.

6-19 Optochin susceptibility test. Colonies of *Streptococcus pneumoniae* are inhibited by the antimicrobic optochin (ethylhydrocupreine hydrochloride) contained in the paper disk applied to the surface of an inoculated 5% sheep blood agar plate. A zone of greater than or equal to 14 mm in diameter is presumptive identification for *Streptococcus pneumoniae*. No zone of inhibition is consistent with viridans streptococci. Zones of less than 14 mm in diameter are questionable and should be confirmed with the bile solubility test.

Bile Esculin Hydrolysis

Both enterococci and non enterococcal sp. of group D are able to hydrolyze esculin in the agar slant , the slant is blacken after (24-48 h).

6-28 Bile esculin slant. *Streptococcus bovis* and the enterococci can grow in the presence of 40% bile and can hydrolyze esculin to esculetin. Esculetin and ferric citrate form a black complex in the agar, which contains 40% oxgall. A negative slant remains tan colored (no color change) compared to black colored slant (positive shown here).

Characteristics of *Enterococcus:*

1- Hydrolysis of esculin.

2- Growth at 45Co

3- presence of 40% bile

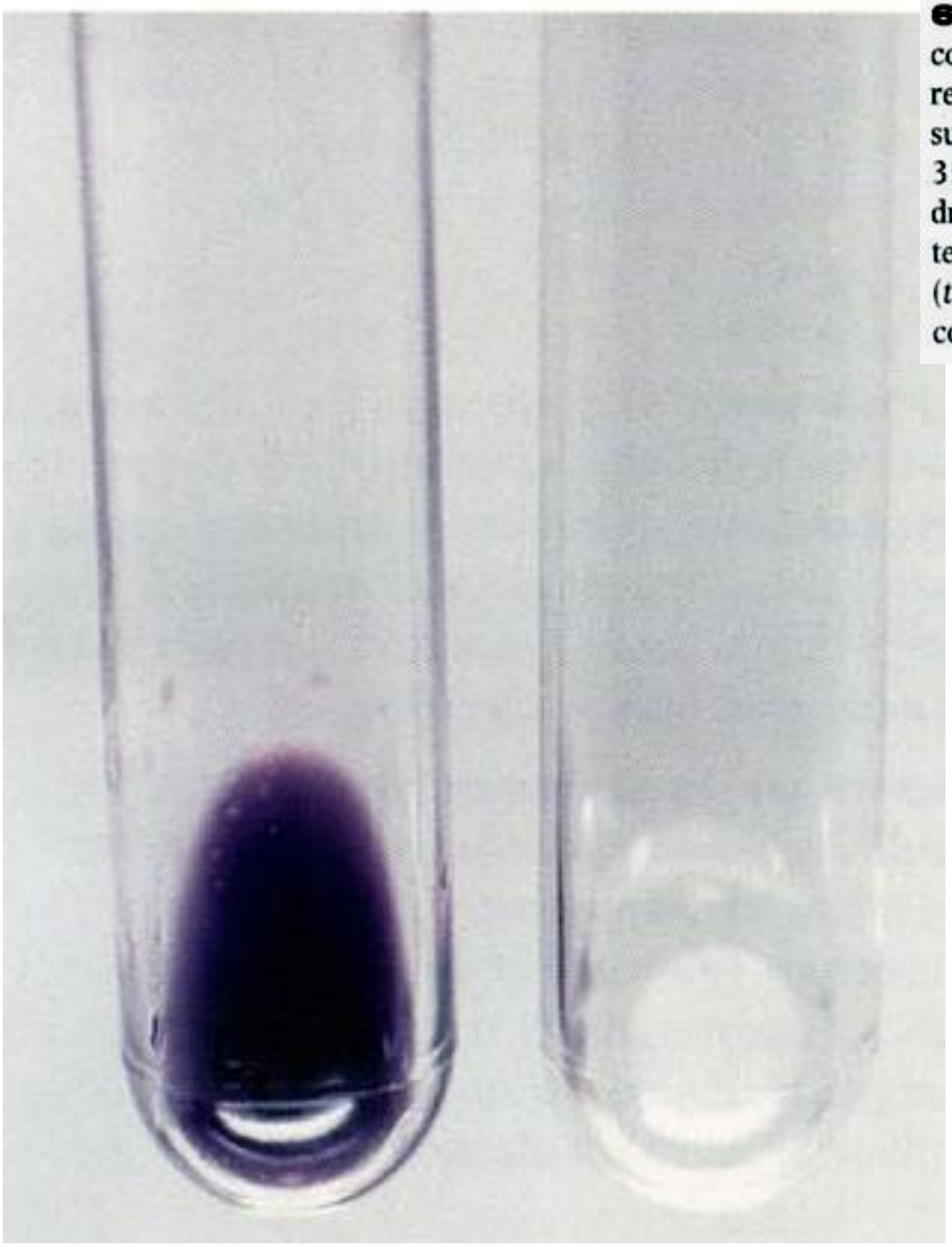
4- Tolerance to 6.5% NaCl

Table(3) :Differential characters of alpha- hemolytic streptococci

Character	*S.pnumoniae*	*Viridans strep.*
Morphology	*Ovoid or lanceolate (diplococci)*	*Short or long chains of rounded cocci*
Capsule	Present in 90%	*Present in 10%*
Optochin sensitivity	sensitive	*Resistant*
Bile solubility	+	–
Virulence in mice	+	–

Table(4): Differential characters of Beta- hemolytic streptococci.

Character	*S. pyogenes(A)*	*S.agalactiae(B)*
Morphology	*Short or long chains*	*Short or long chains*
Bacitracin sensitivity	+	–
Hydrolysis of sodium hippurate	–	+
Growth on Columbia agar	–	+



6-14 Hippurate hydrolysis test. Group B streptococci and some enterococci can hydrolyze sodium hippurate, resulting in the formation of glycine and sodium benzoate. A suspension of the microorganism is incubated for 2 hours at 35°C in a hippurate solution and then the indicator, ninhydrin, is added. Deamination of glycine, if it is present, is detected by the development of a purple color within 10 minutes (*tube on the left*). A negative reaction (*tube on the right*) remains colorless.

Oral Streptococci

The facultative an aerobes Streptococci are the largest group of bacteria isolated from the oral cavity.

Include *S.sanguis, S.salivarus, S.oralis, S.mutans* found in .

1- tooth surface.

2 - epithelial surface.

3- smooth surface dental decay.

The bacterial infections of the tissue surrounding the teeth cause inflammation of the gingival, periodontal ligament, cementum, and alveolar bone.

Oral Streptococci possess enzymes called glucosyl transferase (GTFS) which are able to break down sucrose into glucose and fructose and utilize the glucose moiety in the synthesis of glucan polymers, or glucan.

Some of the glucan synthesized by S.mutans is water insoluble (mutan) and contributes the organism's ability to colonize on tooth surfaces.

The S. mutans use of sucrose and other carbohydrates as energy source which leads to acid production which can promote enamel demineralization.

Colony Morphology

- On blood agar plates (horse blood) Alpha or gamma. Hemolysis, white shiny to smooth translucent.
- On Mitis-Salivarius agar (M-S) Agar is differential medium containing dyes, nutrients, 5% sucrose and growth inhibitors for organisms other than Streptococci.

S. mutans:- pin point to medium size, gray to light or medium blue, the colony is soft to the touch.

S. sanguis:- pin point to medium, translucent to dark blue, shiny colonies are very hard and rubbery to the touch of an inoculating needle.

S. salivarius:- The gum-drop colony, large, circular, entire, pulvinate to highly raised, very mucoid, blue. soft to the touch.

S. oralis:- small to medium , circular, dark blue to opaque black, may be rubbery or soft to the touch. Depressed, flat to slightly raised.

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