

# STREPTOCOCCI

Streptococci are Gram-positive, nonmotile, nonsporeforming, catalase-negative cocci that occur in pairs or chains. Older cultures may lose their Gram-positive character. Most streptococci are facultative anaerobes, and some are obligate (strict) anaerobes. Most require enriched media (blood agar).

Streptococci are subdivided into groups by antibodies that recognize surface antigens (Fig. 11). These groups may include one or more species. Serologic grouping is based on antigenic differences in cell wall carbohydrates (groups A to V), in cell wall pili-associated protein, and in the polysaccharide capsule in group B streptococci. Rebecca Lancefield developed the serologic classification scheme in 1933. **β-hemolytic** strains possess group-specific cell wall antigens, most of which are carbohydrates. These antigens can be detected by immunologic assays and have been useful for the rapid identification of some important streptococcal pathogens. The most important groupable streptococci are A, B and D. Among the groupable streptococci, infectious disease (particularly pharyngitis) is caused by group A. Group A streptococci have a hyaluronic acid capsule. *Streptococcus pneumoniae* (a major cause of human pneumonia) and *Streptococcus mutans* and other so-called viridans streptococci (among the causes of dental caries) do not possess group antigen. *Streptococcus pneumoniae* has a polysaccharide capsule that acts as a virulence factor for the organism; more than 90 different serotypes are known, and these types differ in virulence.

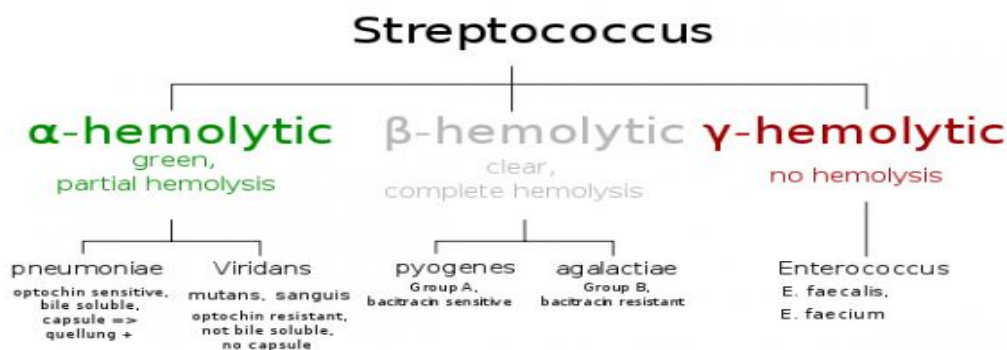


Fig. 1 Streptococci - classification.

Group A streptococci causes:

- Strep throat - a sore, red throat, sometimes with white spots on the tonsils
- Scarlet fever - an illness that follows strep throat. It causes a red rash on the body.
- Impetigo - a skin infection
- Toxic shock syndrome
- Cellulitis and necrotizing fasciitis (flesh-eating disease)

**Rheumatic fever** is a nonsuppurative complication of *S. pyogenes* pharyngitis. Rheumatic fever is an inflammatory disease affecting primarily the heart and joints. Although severe, it can take an extended period of time to develop. The mechanism of chronic immunopathology of rheumatic fever is not resolved. M protein cross-reacts with heart myosin leading to autoimmunity. Also the group A streptococcal cell wall is highly resistant to degradation in the host. These antigens persist for months *in vivo* and experimentally elicit diseases that resemble rheumatic arthritis and carditis. Rheumatic arthritis should not be confused with the most common rheumatic disease - rheumatoid arthritis. Early termination of throat infections with penicillin therapy decreases the incidence of the subsequent development of rheumatic carditis. **Acute glomerulonephritis** is an immune complex disease of the kidney.

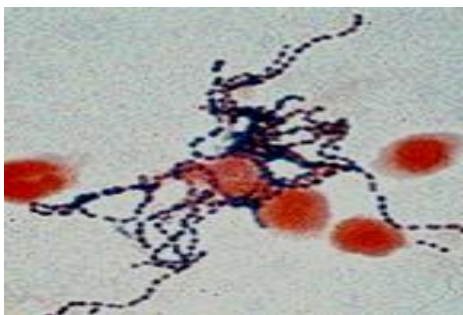
**Group B streptococci** can cause blood infections, pneumonia and meningitis in newborns. Adults can also get group B strep infections, especially if they are elderly or already have health problems. Strep B can cause urinary tract infections, blood infections, skin infections and pneumonia in adults.

**Group D streptococci** is now classified as an Enterococcus. Enterococci are distantly related to other streptococci and have been moved into the genus *Enterococcus*; the most commonly isolated are *E. (S<sup>1</sup>.) faecalis* and *E. (S.) faecium*. *E. (S.) faecalis* can cause nosocomial infections, urinary tract infections, bacteremia, endocarditis, meningitis, and can be found in wound infections along with many other bacteria.

*Streptococcus pneumoniae* causes pneumonia, acute sinusitis, otitis media, meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, and brain abscess. *S. pneumoniae* is the most common cause of bacterial meningitis in adults and children, and is one of the top two isolates found in ear infection, otitis media.

## STREPTOCOCCI - GRAM STAIN

The genus *Streptococcus* is a diverse collection of **Gram-positive cocci** typically arranged in **pairs** or **chains** (in contrast to the clusters formed by *Staphylococcus*) (Fig. 12). *Streptococcus pneumoniae* are lancet shaped (ovoid) cocci in short chains, diplococci and single cocci (Fig 13).



**Fig. 2 *S. pyogenes* - Gram stain.**

---

<sup>1</sup> S. - streptococcus

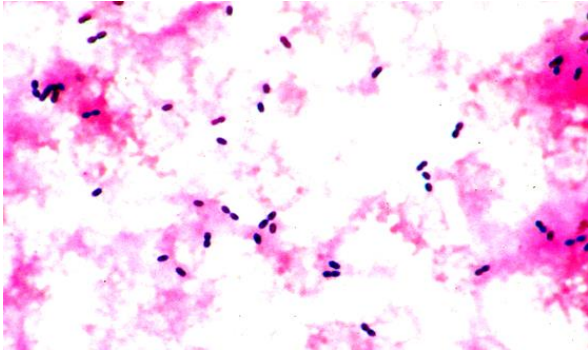


Fig. 3 *S. pneumoniae* - Gram stain.

## STREPTOCOCCI - BLOOD AGAR CULTURE

Most species of streptococci are facultative anaerobes, and some grow only in an atmosphere enhanced with carbon dioxide (capnophilic growth). The classification of species within the genus is complicated because three different schemes are used:

- **hemolytic patterns:** complete ( $\beta$ ) hemolysis, incomplete ( $\alpha$ ) hemolysis, and no ( $\gamma$ ) hemolysis;
- **serologic properties:** Lancefield groupings (originally A to W);
- **biochemical** (physiologic) properties.

### HEMOLYTIC PATTERNS

**Streptococci** are divided into three groups (Fig. 11):

- $\beta$ -hemolytic streptococci**, which are classified by Lancefield grouping,
- $\alpha$ -hemolytic and  $\gamma$ -hemolytic streptococci**, which are classified by biochemical testing.

#### Alpha-hemolysis

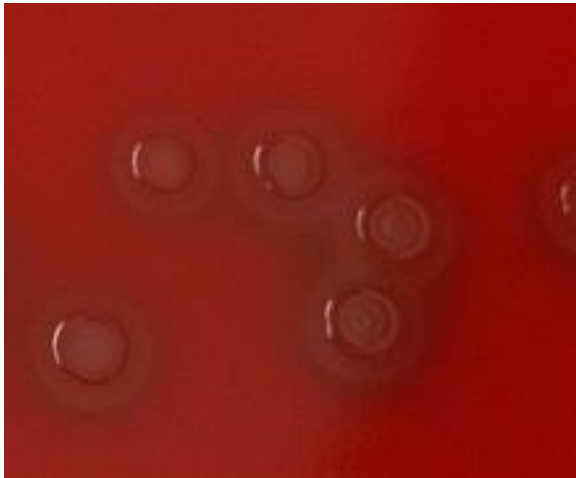
*Streptococcus pneumoniae*, *Streptococcus salivarius*, *viridans* are referred to collectively as **viridans streptococci**, a name derived from *viridis* (Latin for "green"), referring to the green pigment formed by the partial,  $\alpha$ -hemolysis of blood agar. Encapsulated, virulent strains of *S. pneumoniae* often forming highly mucoid, glistening colonies (production of capsular polysaccharide) surrounded by a zone of  $\alpha$ -hemolysis.

When  **$\alpha$ -hemolysis** is present, the agar under the colony is dark and greenish. *Streptococcus pneumoniae* and a group of oral streptococci (*Streptococcus viridans* or viridans streptococci) display alpha hemolysis. This is sometimes called *green hemolysis* because of the color change in the agar.

Other synonymous terms are *incomplete hemolysis* and *partial hemolysis*. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin.

Alpha-hemolytic colonies with depressions in their centers are characteristic of pneumococci

(Fig. 14). Some strains produce high amounts of capsular polysaccharide which gives glistening appearance.

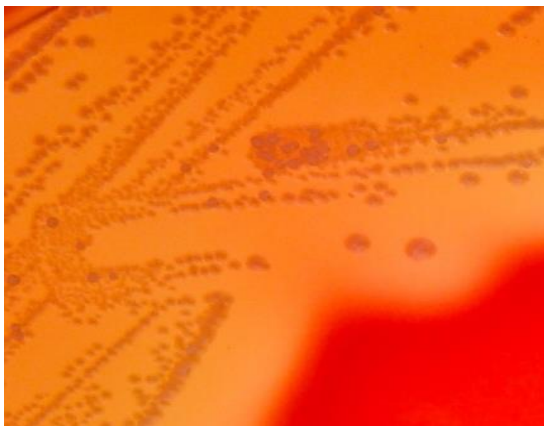


**Fig. 4** *Streptococcus pneumoniae* – alfa hemolysis.

### **Beta hemolysis**

---

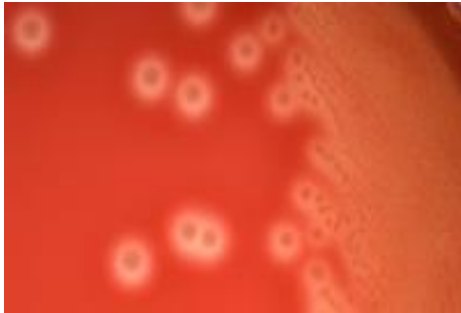
*Streptococcus pyogenes*, or **Group A** beta-hemolytic **Streptococci (GAS)**, and *Streptococcus agalactiae*, or **Group B** beta-hemolytic **Streptococci (GBS)** blood agar cultures display beta hemolysis (Fig. 15, Fig. 16). **Beta hemolysis** ( $\beta$ -hemolysis), sometimes called *complete hemolysis*, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent.



**Fig. 5** *Streptococcus pyogenes* - beta hemolysis.

Streptolysin, an exotoxin, is produced by the bacteria which causes the complete lysis of red blood cells. Streptolysin O is oxygen-sensitive cytotoxin, secreted by most GAS, and interacts with cholesterol in the membrane of eukaryotic cells (mainly red and white blood cells, macrophages, and platelets), and usually results in  $\beta$ -hemolysis under the surface of blood

agar. Colonies of group B streptococci often have less pronounced zones of beta-hemolysis than do other beta-hemolytic streptococci (Fig. 16).



**Fig. 6** *Streptococcus agalactiae* - beta hemolysis.

### **Gamma hemolysis**

---

If an organism does not induce hemolysis, the agar under and around the colony is unchanged, and the organism is called *non-hemolytic* or said to display **gamma hemolysis** ( $\gamma$ -hemolysis). *Enterococcus faecalis* (formerly called Group D Streptococci) displays gamma hemolysis.



**Fig. 7** *Enterococcus faecalis* - gamma hemolysis.

*E. faecalis* typically exhibits gamma-hemolysis on blood agar, but some strains are alpha-hemolytic or even beta-hemolytic (a plasmid-encoded hemolysin, called the cytolysin).

## **STREPTOCOCCI - SEROLOGIC PROPERTIES TESTING**

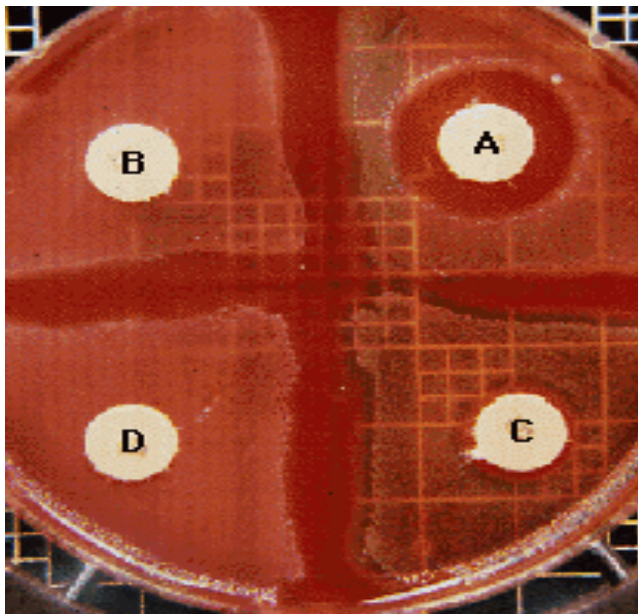
### **Bacitracin sensitivity test ( $\beta$ -hemolytic streptococci Group A, GAS)**

---

**Bacitracin** is a polypeptide antibiotic interfering with the synthesis of peptidoglycan, a unique chemical fabric bacteria include in their cell walls. Bacitracin is common in topical ointments or inhaled into the lungs, but is very rarely given by injection. In a clinical

laboratory, bacitracin is useful in helping identify **Streptococci** and other Gram-positive bacteria. Two concentrations of bacitracin-impregnated disks are made for this purpose, with each being useful in distinguishing among select groups of Gram-positive cocci. Different types of bacteria have different degrees of susceptibility to bacitracin. This test determines whether the bacterium is either sensitive (susceptible) to bacitracin or resistant to the drug. Knowledge about bacitracin susceptibility is valuable in identification of Gram positive cocci, some of which are susceptible and others of which are resistant.

**Principle:** Bacitracin test is used to determine the effect of a small amount of bacitracin (0.04 U<sup>2</sup>) on an organism. *Streptococcus pyogenes* (GAS) is inhibited by the small amount of bacitracin in the disk (visible zone of inhibition of growth, Fig. 18); other beta-hemolytic streptococci usually are not.



**Fig. 8 Bacitracin sensitivity test.**

### CAMP test ( $\beta$ -hemolytic streptococci Group B, GBS)

*S. agalactiae* is the only species that has the group B antigen. This organism was first recognized as a cause of puerperal sepsis. Although this disease is now relatively uncommon, *S. agalactiae* has become better known as an important cause of septicemia, pneumonia, and meningitis in newborn children, as well as a cause of serious disease in adults. **CAMP** - it is an acronym for "Christie, Atkins, Munch-Petersen", for the three researchers who discovered the phenomenon. The CAMP test is a test to identify Group B  $\beta$ -hemolytic streptococci based on their formation of a substance (CAMP factor) that enlarges the area of hemolysis formed by  $\beta$ -hemolysin from *Staphylococcus aureus*. It can be used to identify *Streptococcus agalactiae* (GBS) Though not strongly beta-hemolytic on its own, it presents with a wedge-shape in the presence of *Staphylococcus aureus*.

---

<sup>2</sup> U - unit



**Principle:** The majority of group B streptococci produce a diffusible extracellular protein (CAMP factor) that acts synergistically with staphylococcal beta lysin to lyse erythrocytes.



**Fig. 9 CAMP test - positive result.**

CAMP test showing the arrow-shaped zone of enhanced hemolysis (positive result) of *Streptococcus agalactiae* (group B) and the negative result of *Streptococcus pyogenes* (group A) when tested against *Staphylococcus aureus* (Fig 19, Fig 20).



**Fig. 10 CAMP test - positive (up) and negative (down) result.**

## **STREPTOCOCCI - PHYSIOLOGIC (BIOCHEMICAL) PROPERTIES TESTING**

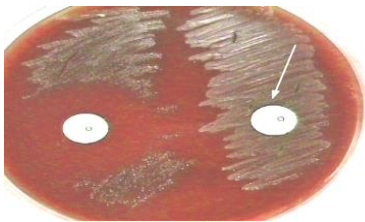
*Streptococcus pneumoniae*, *Streptococcus salivarius*, *Streptococcus viridans* are referred to collectively as **viridans streptococci**, a name derived from *viridis* (Latin for "green"), referring to the green pigment formed by the partial hemolysis of blood agar. The  $\alpha$ -hemolytic (viridans) streptococci are classified by biochemical testing.

### **Optochin test**

---

**Optochin** (or ethylhydrocupreine) is a chemical used in cell culture techniques for the of *Streptococcus pneumoniae*, which is optochin-sensitive (positive result), from other alpha-hemolytic streptococci such as *Streptococcus viridans* which are resistant. Optochin Differentiation Disks are recommended for use in the presumptive identification of

*Streptococcus pneumoniae* from other alpha-hemolytic streptococci. The growth of bacteria that are optochin sensitive will be inhibited around optochin disc, while the growth of bacteria that are optochin resistant will not be affected. A zone of inhibition of  $\geq 15$  mm around the disk is considered a susceptible result (Fig. 21). In vitro a solution of as little as 1: 10,000,000 may be inhibitory to the pneumococcus, and 1: 500,000 is bactericidal. Strains of other viridans streptococci have been found to be resistant to optochin in laboratory testing.

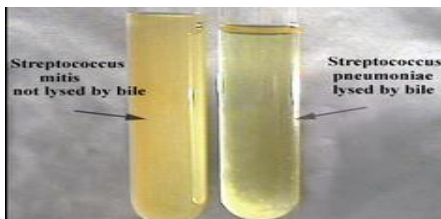


**Fig. 11 Optochin Test - positive result (left).**

### **Bile solubility test**

---

The bile (sodium deoxycholate) solubility test distinguishes *Streptococcus pneumoniae* from all other alpha-hemolytic (viridans) streptococci. *Streptococcus pneumoniae* is bile soluble whereas all other alpha-hemolytic streptococci are bile resistant. Sodium deoxycholate (2% in water) will lyse the pneumococcal cell wall. A clearing of the turbidity in the bile tube indicates a positive test (Fig. 22).



**Fig. 12 Bile solubility test - positive result (right).**

## **ANTISTREPTOLYSIN O (ASO)**

**Anti-streptolysin O (ASO)** is the antibody made against streptolysin O, an immunogenic, oxygen-labile hemolytic toxin produced by most strains of group A and many strains of groups C and G streptococci. The O in the name stands for *oxygen-labile*; the other related toxin being oxygen-stable streptolysin-S. When the body is infected with streptococci, it produces antibodies against the various antigens that the streptococci produce. A raised or rising levels can indicate past or present infection. Historically it was one of the first bacterial markers used for diagnosis and follow up of rheumatic fever or scarlet fever. Its importance in this regard has not diminished. Since these antibodies are produced as a delayed antibody reaction to the above mentioned bacteria, there is no normal value. The presence of these



antibodies indicates an exposure to these bacteria. However, as many people are exposed to these bacteria and remain asymptomatic, the presence of ASO does not indicate disease.

Acceptable values, where there is no clinical suspicion of rheumatism are as follows:

- Adults: less than 200 units
- Children: less than 400 units

This titre has a significance only if it is greatly elevated (>200), or if a rise in titre can be demonstrated in paired blood samples taken days apart. The antibody levels begin to rise after 1 to 3 weeks of streptococcal infection, peaks in 3 to 5 weeks and falls back to insignificant levels in 6 months. Values need to be correlated with a clinical diagnosis.

## C-REACTIVE PROTEIN (CRP)

C-reactive protein (CRP) is found in the blood and is a response to inflammation in the body. It can also be an indicator of the presence of infection, trauma or serious illness. Chronic inflammation can keep CRP levels elevated, which can increase the risk of cardiovascular conditions such as heart attacks or stroke. C-reactive protein is produced by the liver. The level of CRP rises when there is inflammation throughout the body. It is not a specific test.

---

### SOURCES:

Patrick R. Murray, PhD, Ken S. Rosenthal, PhD and Michael A. Pfaller, MD: *Medical Microbiology*, 7th Edition, Elsevier Health Sciences, 2012; ISBN: 978-0-323-08692-9

Samuel Baron *Medical Microbiology*, 4th edition, University of Texas Medical Branch at Galveston, Galveston, Texas Galveston (TX): University of Texas Medical Branch at Galveston; 1996. ISBN-10: 0-9631172-1-1

Alvin Fox *STREPTOCOCCI GROUPS A, B, D AND OTHERS ENTEROCOCCUS FAECALIS*, In: *Microbiology and Immunology On-line*, Hunt, R.C. editor.

Antistreptococcal antibody titres. gnotebook. URL: <http://www.gnotebook.co.uk/cache/-1630535637.htm>

Danchin M, Carlin J, Devenish W, Nolan T, Carapetis J (2005). "New normal ranges of antistreptolysin O and antideoxyribonuclease B titres for Australian children". *J Paediatr Child Health* **41** (11): 583–6. doi:10.1111/j.1440-1754.2005.00726.x.

C-reactive protein. (2013, January). Retrieved from <http://my.clevelandclinic.org/services/heart/diagnostics-testing/laboratory-tests/blood-tests-to-determine-risk-of-coronary-artery-disease>

Mayo Clinic Staff. (2014, December 12). C-reactive protein test. Retrieved from <http://www.mayoclinic.com/health/c-reactive-protein/my01018>

Ridker, P. M., Glynn, R. J., & Hennekens, C. J. (1998, May 26). C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction.

*Circulation*, 97(20), 2007–2011. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9610529>

PMID 16398843.<http://microbeonline.com/optochin-test-principle-procedure-expected-results-and-quality-control/>

<http://www.cdc.gov/meningitis/lab-manual/chpt08-id-characterization-streppneumo.pdf>

<http://www.microbiologybook.org/fox/streptococci.htm>

<http://www.microbiologyinpictures.com/bacteria%20photos/streptococcus%20pneumoniae%20photos/STPN10.html>

<http://www.microbiologyinpictures.com/streptococcus%20agalactiae.html>

<http://microbeonline.com/camp-test-principle-procedure-results/>  
<http://microbeonline.com/microbiologic-methods-for-diagnosis-of-streptococcus-pneumoniae/>

<http://emedicine.medscape.com/article/225811-clinical>

