

## Diagnosis of Streptococci

### Group A streptococci

#### **LABORATORY FEATURES**

*Specimens:* Include a throat swab (avoiding saliva contamination) or swabs of pus and serous fluid depending on the site of infection, and blood for culture. Testing for ASO antibody in serum is helpful in diagnosing rheumatic fever.

#### **Morphology**

Streptococci are Gram positive cocci, occurring characteristically in short chains, but also in pairs and singly. Long chains are formed in fluid cultures. The organisms are non-motile. Some strains are capsulated.

**Blood agar:** *S. pyogenes* produces *beta*-haemolytic colonies

**Catalase test** can be used to differentiate streptococci (negative) from staphylococci (positive)

**Antibiotic sensitivity: Sensitivity to bacitracin**

**PYR (pyrrolidonyl) test:** This detects pyrrolidonyl peptidase enzyme activity. Besides *S. pyogenes*, *Enterococcus* species and occasionally streptococci belonging to groups C and G are also PYR positive.

The substrate for the PYR test is L-naphthylamide- $\beta$ -naphthylamide which is hydrolyzed by a specific bacterial aminopeptidase enzyme.

Hydrolysis of the substrate by this enzyme releases free  $\beta$ -naphthylamide, which is detected by the addition of N, N-dimethyl amino-cinnamaldehyde.

1. With a sterile bacteriologic loop, pick up the growth of two to three morphologically similar colonies and emulsify them in the small volume of PYR broth
2. Incubate the tube at 35°C for 4 hours
3. Add one drop of PYR reagent and observe for color change.
4. The reaction should be read and recorded 1 minute after the addition of reagent.

#### **Measurement of ASO antibody in serum**

ASO (anti-streptolysin O) antibody is formed in response to infection with *S. pyogenes* and other streptococci that produce streptolysin O (some Group C and G strains). Following infection, most patients show a high

titre of ASO antibody in excess of 200 IU/ml). Measurement of ASO antibody titre is important in the investigation of post-streptococcal diseases, particularly rheumatic fever which usually develops 2–3 weeks or more after streptococcal sore throat when it is often not possible to isolate *S. pyogenes* in culture.

### ***Streptococcus agalactiae***

#### **Culture**

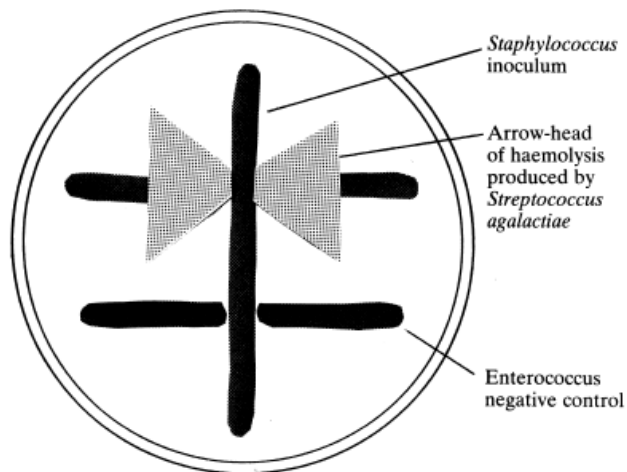
**Blood agar:** Most strains of *S. agalactiae* produce grey mucoid colonies about 2 mm in diameter, surrounded by a small zone of *beta*-haemolysis. About 5% of strains are nonhaemolytic. Placing discs of penicillin and gentamicin on the plate can help to identify these strains (penicillin sensitive, gentamicin resistant).

**MacConkey agar:** Most strains grow on this medium.

**Neomycin blood agar:** A useful selective medium for isolating *S. agalactiae* from urogenital specimens.

#### **CAMP (Christie, Atkins, Munch, Peterson) test to identify presumptively *S. agalactiae***

This test requires the use of a *beta*-lysin producing strain of *S. aureus* to detect the CAMP factor produced by *S. agalactiae* (which is an extracellular diffusible protein). This protein interacts with the staphylococcal *beta*-lysin on sheep (or ox) blood agar producing enhanced haemolysis.



**Bile aesculin stope:** *S. agalactiae* does not hydrolyse aesculin. It is able to grow on bile agar. Group A *Streptococcus pyogenes* gives a variable aesculin hydrolysis reaction and does not grow on bile agar. Group D streptococci hydrolyse aesculin and can grow on bile agar.

#### **Hippurate hydrolysis test**

*S. agalactiae* hydrolyzes hippurate. The test can be inexpensively and rapidly performed using a saline suspension of the test organism and a Diagnostica **hippurate hydrolysis tablet**, and **ninhydrin 3.5% reagent**

### ***S. pneumoniae***

## **LABORATORY FEATURES**

*Specimens:* Depending on the site of infection, specimens include sputum, exudate, blood for culture, and cerebrospinal fluid.

### **Morphology**

Gram positive elongated (lanceolate) diplococcus. It also forms short chains, particularly following culture. Pneumococci are nonmotile and capsulated.

### **Culture**

**Blood agar:** Following overnight incubation. *S. pneumoniae* forms translucent or mucoid colonies, 1–2 mm in diameter, show *alpha*-haemolysis. In young cultures the colonies are raised but later become flattened with raised edges, giving them a ringed appearance ('draughtsmen').

### **Optochin sensitivity**

Pneumococci are sensitive to optochin

### **Bile solubility test**

Add a loopful of 2% sodium deoxycholate reagent (pH.7.0) directly on a culture plate by touching *alpha*-haemolytic colonies, incubating the plate at 35–37 °C for 30 minutes, and examining for lysis (disappearance of the colony, indicating *S. pneumoniae*).

### **Direct detection of pneumococcal antigen in body fluid**

Rapid latex and coagglutination tests are available to detect capsular pneumococcal antigen in c.s.f., pleural fluid, serum and urine.

### **Viridance streptococci**

The following are the main features which differentiate *S. pneumoniae* from viridans streptococci:

<i>Features</i>	<i>S. pneumoniae</i>	<i>Viridans streptococci</i>
Haemolysis	<i>Alpha</i>	<i>Alpha, beta, non-haemolytic</i>
Optochin	Sensitive	Resistant
Bile Solubility	Positive	Negative

## ***Enterococcus***

### **Morphology**

Gram positive cocci, occurring in pairs or short chains. They are non-capsulate and the majority are non-motile.

### **Culture**

Enterococci are aerobic organisms capable of growing over a wide temperature range, 10–45 °C.

***Blood agar:*** Enterococci are mainly nonhaemolytic but some strains show *alpha* or *beta*-haemolysis.

***MacConkey and CLED agar:*** *E. faecalis* ferments lactose, producing small dark-red magenta colonies on MacConkey agar and small yellow colonies on CLED (cysteine lactose electrolyte-deficient) agar.

*Enterococcus* species are also able to grow in the presence of 6.5% sodium chloride and 40% bile. When grown on media containing aesculin, enterococci hydrolyze the aesculin, producing black colonies.