Diagnosis of Streptococci

S. pneumoniae

LABORATORY FEATURES

Specimens: Depending on the site of infection, specimens include sputum, exudates , blood for culture, and cerebrospinal fluid. Pneumococci are frequently found as normal flora of the nasopharynx of healthy carriers .

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.

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*).



Bile solubility test

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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:

Features	S. pneumoniae	Viridans streptococci
Haemolysis	Alpha	<i>Alpha, beta,</i> non-haemolytic
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.

Bile aesculin test:

Bile Esculin Agar is an undefined, selective, and differential medium

In this test, when esculin molecules are split, esculetin reacts with the Fe3+ from the ferric citrate and forms a dark brown precipitate. This precipitate darkens the medium surrounding the growth. An organism that darkens the medium even slightly is Bile Esculin-positive. An organism that does not darken the medium is negative.



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.