Practical No.10

Streptococcus pneumoniae

S. pneumoniae is a Gram-positive, lanceolate shaped coccus that is usually seen in pairs of cocci (diplococci). It is non motile and non spore-forming. It lacks catalase and ferments glucose to lactic acid. It doesn't display an M protein, but it hydrolyzes inulin, and has a cell wall that contains a thick peptidoglycan layer as well as teichoic acids.

S. pneumoniae causes a variety of diseases including: otitis media, sinusitis, pneumonia, meningitis, and septicemia .

Pneumococcal colonies are surrounded by a greenish zone of alpha -hemolysis. *S. pneumoniae* (pneumococcus) requires elevated CO_2 concentrations for growth. Therefore, isolation of this organism requires incubation in 5% CO_2 or a candle jar for growth. Young *S. pneumoniae* colonies are round, shiny, gray, and domed. As the culture ages, autolysis allows the central portion to sink, yielding a flattened surface or central depression.

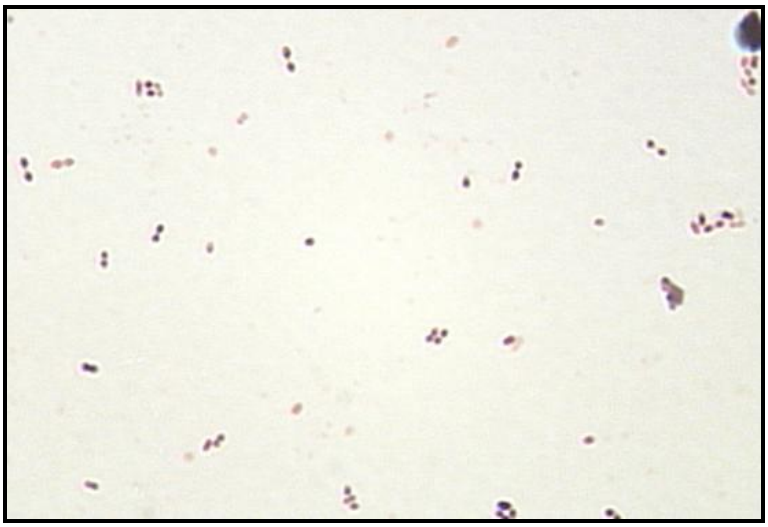
Identification of ***S. pneumonia*** and ***viridans streptococci***

The alpha-hemolytic streptococci, or viridans (green) group (e.g. *S. mitis, S. sanguis, S. mutans, S. salivarius*), are normal inhabitants of the mouth, nasopharynx, and respiratory tract. Alpha-hemolytic streptococci may be isolated by blood culture in cases of bacterial endocarditis.

Specimen sputum, blood, throat swab, ear swab, CSF

1- Gram stain of *Streptococcus pneumoniae* and viridans Streptococci.

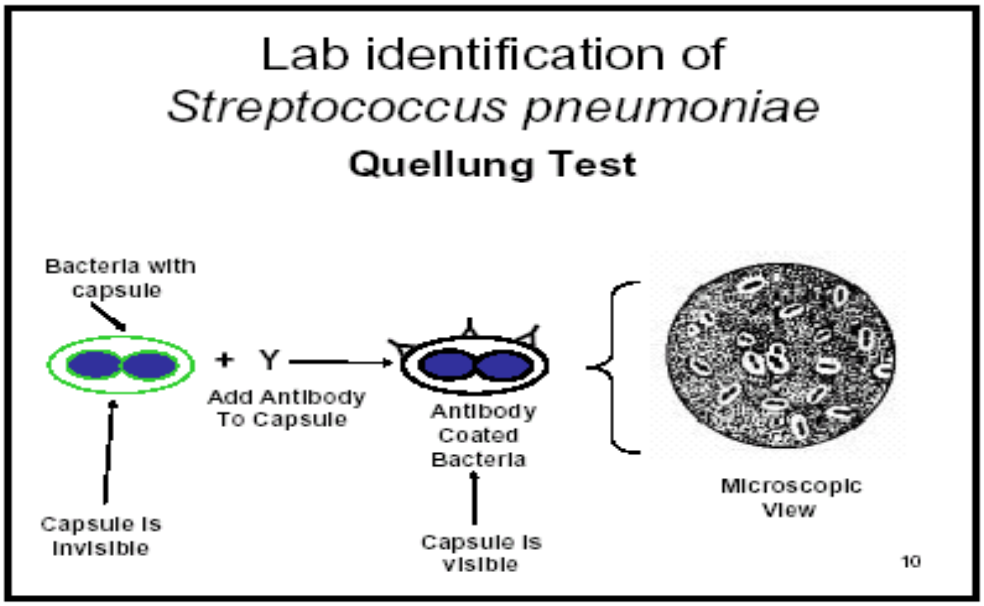
Gram stain of *S. pneumoniae* and a viridans streptococcus obtained from the Blood agar plate, notice the cell morphology and arrangement:



Strept. pneumoniae gram-positive diplococci. Notice the characteristic elongated shape of the **diplococci**.

2- The Quellung reaction to differentiate *S. pneumoniae* from viridans streptococci.

The **Quellung reaction** (capsular swelling reaction) is a biochemical reaction in which antibodies bind to the capsule of *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* and thus allow them to be visualized under a microscope. If the reaction is positive, the capsule becomes opaque and appears to enlarge.

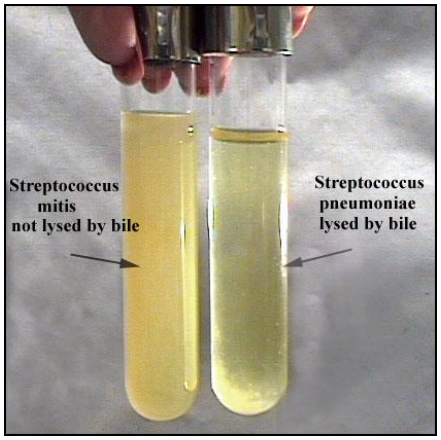


1- Bile solubility test to differentiate *S. pneumoniae* from viridans streptococci.

The bile solubility test may be performed by adding the sodium desoxycholate solution to a cell suspension or directly to colonies on a blood agar plate.

Procedure: The bile solubility test is performed by adding a bile-salt solution to an established broth or blood-agar culture of the organism in question. A positive result in broth culture is obtained by noting visible clearing of the culture's turbidity, as compared to a control tube, after addition of the bile salt solution and re-incubation for up to 3 hours.

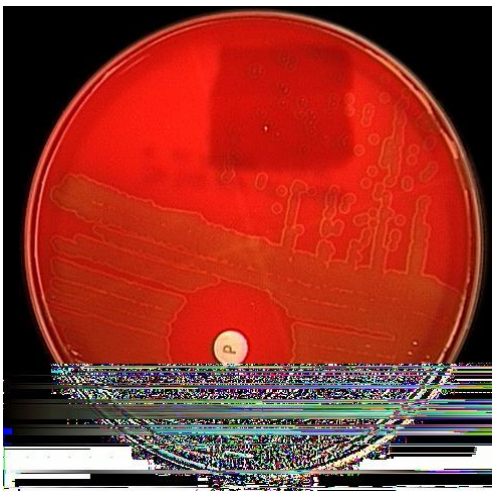
This test is most easily performed by placing a drop of bile solution (10% sodium deoxycholate) on suspected colonies on solid media. In 5-10 minutes, colonies of *Pneumococci* will lyse while those of alpha hemolytic *Streptococci* will not. The test can be performed by adding the reagent to an actively growing broth culture and determining whether it will clear in 5-10 minutes.



Bile solubility test By adding 5ml of 10% sodium deoxycholate to saline suspension of culture and incubate for 15 minute).

2- Optochin-Susceptibility of *S. pneumoniae,* but not of a Viridans Streptococcus:

The Optochin disc is used in most laboratories for differentiation. Discs impregnated with optochin (ethylhydrocupreine hydrochloride), when placed on a freshly inoculated blood agar plate, will inhibit the growth of pneumococci but allow other □ -hemolytic streptococci to grow normally. When colonies suspected as pneumococci are seen, several colonies are streaked on a fresh blood agar plate and an Optochin disc is placed on the inoculated area of the first streak quadrant prior to incubation (as was done for bacitracin for the Group A *Streptococcus*). The plate is then incubated for 18-24 hours. A zone of inhibition 14 mm or greater is read as a positive test; a zone of 6 to 14 mm is a questionable result.



Observe the demonstration plate for Optochin susceptibility of *S. pneumoniae*

3- Inulin fermentation test; Inulin fermentation test; in the presence of the pneumococci there will be a change in the colour of the media due to acid formation**.**

Procedure;

-Inoculate a test tube containing nutrient broth + inulin + the organism, (red colour).

-Then incubate over night, if the colour remains red the organism is Viridans Streptococcus,

If the organism has fermented the inulin resulting in acid production. The acid lowers the pH of the media and the indicator (phenol red) turns yellow.

Observe for the presence of a yellow color (center tube), indicative of acid formation from the fermentation of inulin. Red indicates no inulin fermentation (left tube; right tube is uninoculated control).

Differentiation of alpha and non-hemolytic streptococci

Category	**Viridans streptococci**	**Streptococcus pneumoniae**
Solubility in bile	Insoluble	Soluble
Fermentation of inulin	Not a fermenter	Fermenter with acid production
Sensitivity to optochin	Not sensitive	Sensitive
Pathogenicity to mice	Non pathogenic	Pathogenic
Quellung test	Negative	Positive