BILE SOLUBILITY REAGENT

- For in vitro use only - Catalogue No. RB60

Our Bile Solubility Reagent can be used to differentiate bile-soluble *Streptococcus pneumoniae* from other bile-insoluble α-hemolytic streptococci.

S. pneumoniae possesses an autolytic enzyme that causes the organism to breakdown its own cell wall causing lysis of the cell. In the presence of the bile salt, sodium deoxycholate, the autolytic process is accelerated. On a solid medium, colonies of *S. pneumoniae* disintegrate and disappear, or if growth is suspended in saline the turbid solution exhibits a clearing effect. In both cases, the reactions are classified as soluble. Bile (Oxgall) and other bile salts, such as sodium taurocholate can also be used to perform the test but often give variable results. Other α-hemolytic streptococci do not possess active autolytic enzymes and will not dissolve in bile, and are classified as bile insoluble.

It should be noted that only 80% of *Streptococcus pneumoniae* strains will lyse completely, and additional tests may be required to differentiate those strains that show only partial or incomplete lysis.

Formulation per 100 mL

Sodium Deoxycholate.....10.0 g
Sterile Deionized Water..... 100.0 mL

Recommended Procedure

Plate Procedure

1. Perform a four-quadrant streak of the test organism onto a blood agar plate to obtain well-isolated colonies. (The test organism should be an α-hemolytic, catalase-negative, gram-positive cocci arranged in chains and this determination should be made prior to performing the test)
2. Incubate plates in an inverted position for 18 to 24 hours at 35°C.
3. Select a characteristic *S. pneumoniae* colony that is well-isolated. Mark the location of the colony on the bottom of the petri dish using an wax pencil or permanent felt marker.
4. Place a loopful or a drop of Bile Solubility Reagent onto the colony of interest.
5. Incubate plate aerobically, agar-side down and lid-side up, at 35°C. Do not invert the plate.
6. After 30 minutes check the plate for appearance of the suspect colony.

Tube Procedure

1. Obtain a pure, overnight culture of the streptococci of interest from a blood agar plate or Todd-Hewitt Broth.
2. Prepare a heavy suspension of the organism in 1.0-mL of physiologic saline solution (Dalynn BS85 or TS85).
3. Add 1 drop of phenol red indicator and, if required, adjust the pH to 7.0 using 0.1 N sodium hydroxide. The solution should appear pink. (This step can be skipped but the pH must be above 6.8 to obtain accurate results)
4. Divide the saline suspension equally into two tubes (0.5-mL per tube). Label one tube test and the other control.
5. Add 0.5-mL (4 drops) of Bile Solubility Reagent to the tube marked test.
6. Add 0.5-mL (4 drops) of sterile physiologic saline to the tube marked control.
7. Gently agitate both tubes to ensure that the suspensions are homogenous.
8. Incubate tubes at 35 to 37°C.
9. Check tubes hourly and make a final interpretation after 3 hours. Observe for clearing of the broth.

Interpretation of Results:

Bile Soluble:
Plate Procedure - Bile-soluble colonies disintegrate and disappear under the drop of reagent. A flattened imprint of the lysed colony may remain and an area of hemolysis may appear at the drop location.

Tube Procedure - A clearing of the turbidity is observed when the test aliquot is compared to the control aliquot. An increase in the viscosity of the suspension may also be observed.

Bile Insoluble:
Plate Procedure - Colonies remain intact and visible.

Tube Procedure - Test aliquot remains turbid and is equivalent to the control aliquot after 3 hours of incubation

- *For the plate procedure some literature recommends the use of a 2% solution of deoxycholate versus our 10%. If desired, the 10% solution can be further diluted using sterile water to obtain the desired 2% concentration*

- *For the plate procedure, sometimes alpha-hemolytic colonies do not dissolve but lift off and float away therefore avoid shaking and unnecessary movement of plates after the reagent is added*

- *For the tube test the pH must be above 6.8 since sodium deoxycholate can form a precipitate in an acid suspension (6.5 and lower) and give false-negative results*

- *Downie reported that a loss of the capsule may alter the susceptibility of S. pneumoniae to lysis by bile salts*

- *α-hemolytic Haemophilus species (H.. influenzae and H. aegypticus) are also bile soluble and the reagent may be used to help differentiate between these species from other bile-insoluble Haemophilus spp.*

Quality Control

Organism	**Expected Results**	
Streptococcus pneumoniae ATCC 6305 / 12344	+ve	Disintegration of colony
Streptococcus mitis ATCC 15909	-ve	Colony stays intact

Storage and Shelf Life

Our Bile Solubility Reagent should be stored at room temperature. At this temperature it has a shelf life of 26 weeks from the date of manufacture.

References

1. Anderson AB, Hart PDA. The lysis of pneumococci by sodium deoxycholate. Lancet 1934; 2:359-60.
2. Hawn CVZ, Beebe E. Rapid method for demonstrating bile solubility of *Diplococcus pneumoniae*. J Bacteriol 1965; 90:549.
3. Branson D. Methods in clinical bacteriology. Springfield, IL: Charles C Thomas, 1972.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol 1. Washington, DC: ASM, 1992.
5. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.
6. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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