

Bile Solubility Test

I. Principle

Frequently the differentiation between *Streptococcus pneumoniae* and the viridans streptococci cannot be based upon gross morphology alone. *S. pneumoniae* lyse when treated with a 10% solution of sodium desoxycholate, while other streptococci and Gram positive cocci are not bile soluble. Lysis occurs because bile soluble organisms contain autolytic amidase that when activated by bile salts cleaves the bond between alanine and muramic acid in the cell wall.

II. Reagents

A. Desoxycholate Reagent (2% sodium desoxycholate). Store at room temperature until stated expiration date.

III. Quality Control

A. Quality control is performed with each new batch, lot, or shipment with the following control organisms:

1. *S. pneumoniae* ATCC 49619 = bile soluble (lysis)
2. *E. faecalis* ATCC 29212 = bile insoluble (no lysis)

B. Controls must display expected results. If controls do not display expected results, quality control must be repeated.

IV. Procedure

A. Using a pipette, dispense one drop on a well-isolated alpha-hemolytic colony on a BAP incubated for 18-24h.

B. Incubate the plate at RT, agar side down for 15 min or at 35°C for 30 min.

C. Examine the area where the reagent was applied for evidence of colony disintegration or lysis.

D. Interpretation

1. Dissolving of the colony is a positive test for *S. pneumoniae*.
2. Colonies remaining intact is a negative test for *S. pneumoniae*.

V. Limitations

A. False negative results may occur when testing cultures older than 18-24h.

B. Occasionally, alpha-hemolytic colonies do not dissolve but merely lift off the surface of the agar, float away, and settle elsewhere on the plate. The plate should be carefully examined for evidence of this.

VI. References

A. Hawn and Beeve, 1965. *J. Bacteriol.* 90:549

B. Pratt-Rippin, K and M Pezzlo, 1992. Identification of aerobic Gram positive bacteria: Bile solubility tests. In: *Clinical Microbiology Procedures Handbook*, H.D. Isenberg (ed.). American Society for Microbiology, Washington, DC. pp 1.20.19-1.20.20.

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