TITLE: Bile Solubility	Revision Date: 20-April-2018	Issue Date: 20-April-2016
Document Number: MIC52900	**Status: Approved**	
Distribution: Microbiology Test Manual	**Page:** 1 of 5	
Approved by: S. Asmussen, Manager of Diagnostic Services	**Signed by:**	

PURPOSE:

Test is used for the presumptive identification and differentiation of *Streptococcus pneumoniae* from other alpha hemolytic streptococci.

INTRODUCTION:

Streptococcus pneumoniae possess an autolytic intracellular enzyme that lyses the organism's own cell wall during cell division. The bile solubility test is based on the observation that *S.pneumoniae* cells visibly lyse when 10% sodium desoxycholate is applied, while other alpha-hemolytic streptococci do not. Two methods can be utilized: tube test or the direct plate method.

SAMPLE INFORMATION:

Type	Catalase Negative, alpha hemolytic Gram positive cocci
Source	Sterile sites
Stability	See expiration date
Storage Requirements	20-25°C, RT
Criteria for rejection and follow up action	Visible signs of deterioration, color change, expired reagent

REAGENTS:

- Bile Solubility Reagent: remel REF:R21206

SUPPLIES:

- test tubes
- 37°C incubator
- Normal saline
- Pipette
- Sterile wooden stick

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

QUALITY CONTROL:

Test each new lot of reagent with positive and negative control:

- Positive control: *Streptococcus pneumoniae* ATCC49619
- Negative control: *Streptococcus salivarius* ATCC13419

PROCEDURE INSTRUCTIONS: TUBE TEST

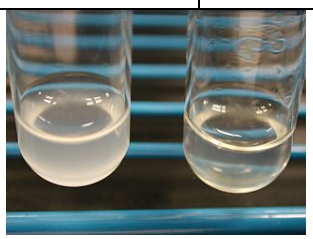
Step	Action
Tube Test for Bile Solubility	
1	Prepare a 1.0mL saline suspension equivalent to a 1.0 McFarland of the test isolate from an 18-24hr culture into a glass test tube – label tube with the accession number and CONTROL. Example: 1234567CTRL
2	Aliquot 0.5mL of suspension from Step 1 into a second glass test tube – label with the accession number and TEST. Example: 1234567TEST
3	To the tube labeled CONTROL – add 0.5mL of sterile saline and gently mix
4	To the tube labeled TEST – add 0.5mL of 10% Bile Solubility reagent and gently mix
5	Incubate tubes at 37°C – examine hourly for clearing in the TEST suspension up to a maximum of 3hrs
6	The control suspension should remain turbid

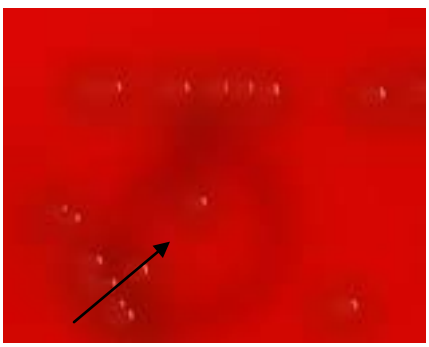
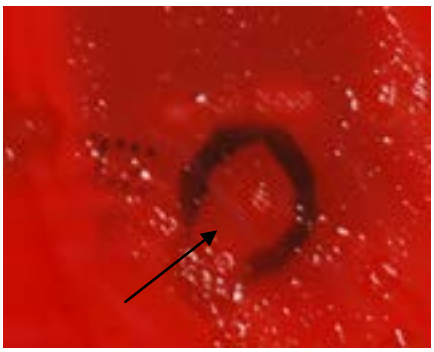
PROCEDURE INSTRUCTIONS: DIRECT PLATE METHOD

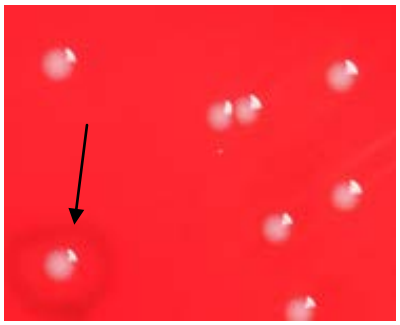
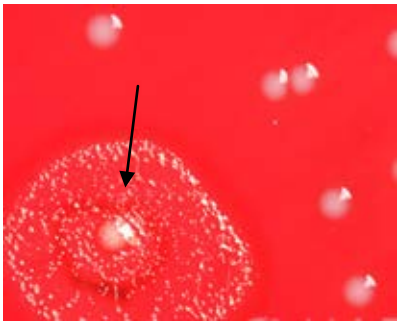
Step	Action
Direct Plate Method for Bile Solubility	
1	Add 1 drop of 10% bile solubility reagent to a well-isolated, 18-24hr colony of the test isolate. To achieve well-isolated colonies, a sub-culture may have to be done.
2	Incubate plate aerobically - agar down and always level, in a 37°C incubator for 30mins. Plate MUST be kept level to prevent the possibility of non-pneumococcal colonies from washing away with the reagent (colonies carried away with reagent is not the same as auto-lysing).
3	Place the lid slightly ajar to allow for evaporation
4	Observe the colony for disintegration or solubility

INTERPRETATION OF RESULTS:

TUBE METHOD	
IF	**THEN**
TEST suspension: clear CONTROL suspension: turbid	Test is POSITIVE; isolate is presumptive *S.pneumoniae*
TEST suspension: turbid CONTROL suspension: turbid	Test is NEGATIVE; isolate is not *S.pneumoniae*
TEST suspension: turbid CONTROL suspension: clear	Test is invalid; repeat testing – probable mislabeling of test tubes

Control  **Test**

DIRECT PLATE METHOD	
IF	**THEN**
Colony has disintegrated	Test is POSITIVE; isolate is presumptive *S.pneumoniae*
Colony remains intact	Test is NEGATIVE; isolate is not *S.pneumoniae*
Before bile salts: 	**After** bile salts: Colony has disintegrated – Test is **POSITIVE** 

Before bile salts:	**After** bile salts: Colony remains intact – Test is **NEGATIVE**
	

REFERENCES:

- Remel. (2012, July). Bile Solubility Reagent (2% and 10% Sodium Desoxycholate).

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	30Dec13	Initial Release	Darrach (A)
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell