Ministry of Health

Document Title	Bile Solubility Test SOP
Document Type	Procedure
Directorate/Institution	The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH)
Targeted Group	Medical laboratories
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Release Date	May 2023
Review Frequency	Three Years

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Date	May 2023	Date	June 2023

Contents Table:

Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

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Acronyms:

BA	Blood agar
ATCC	American Type Culture Collection
SOP	Standard operating procedure

1. **Purpose:**

   This procedure provides instruction how to perform the bile solubility test for the presumptive identification and confirmation of alpha-hemolytic *Streptococcus pneumoniae*.

2. **Scope:**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions:**

   3.1 Alpha-hemolytic: is due to partial destruction of red blood cells on blood agar plate which result in green color around the colonies.

4. **Procedure:**

   4.1. Clinical background:

   Rapid identification of *Streptococcus pneumoniae* recovered from significant clinical isolates (e.g. Blood) can provide information about the source of infectious and guide the clinician to specific therapeutic treatment of the patient. *S. pneumoniae* is a fastidious bacterium, growing best in Blood agar at 35-37°C with ~5-10% CO_2 which can occurs intracellularly or extracellularly as gram-positive lanceolate diplococci, but can also occur as single cocci or in short chains of cocci.

   4.2. Principle of the test:

   This test is used specifically to differentiate between *Streptococcus pneumoniae* (bile soluble) and other α-haemolytic *streptococci* (not bile soluble). This test is used to determine the ability of bacterial cells to lyse in the presence of bile salts, within 30 minutes at 36°C ±1. *S. pneumoniae* possesses an autolytic enzyme, an amidase, which lyses the cell's own wall during division. The addition of bile salts (sodium deoxycholate) activates the autolytic enzyme and the organisms rapidly autolyse. Other α-haemolytic streptococci do not possess such an active system and therefore do not dissolve in bile.

   4.3. Pre – analytical stage:

   4.3.1. Sample:

   4.3.1.1 Sample type: well young growth of isolated colonies.

   4.3.1.2 Sample source: solid culture medium.

4.3.1.3 Amount of sample required, including minimum requirements: 2-3 colonies.

4.3.1.4 Sample stability and storage requirements: 18-24 hours.

4.3.1.5 Criteria for unacceptable samples and follow-up action: Culture older than 24 hours are not acceptable.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
➢ 10% Sodium deoxycholate. ➢ 2% Sodium deoxycholate ➢ Blood Agar.	Test tube. Sterile disposable wire loops 10 µl. Sterile pasture pipette 1-3 ml.	Incubator 35°C ±1. Fridge (Storage: 2-8°C).

4.3.3. Safety precaution:

4.3.3.1 All specimens need to be treated as potentially infectious.

4.3.3.2 Standard procedures for handling of biohazard material must be followed at all times.

4.3.3.3 Universal Precautions must be practiced at all stages of these procedures.

4.3.4. Quality control:

4.3.4.1 Check the expiry dates of all media, reagents and stains before use.

4.3.4.2 All media, reagents, kits, and stains **MUST** be quality controlled before use.

4.3.4.3 Identification tests should be run with appropriate controls.

4.3.4.4 Reagent shall be labelled properly with expiry date once in use.

4.3.4.5 Use known positive and negative control with ATCC strains.

4.3.4.6 Positive control (bile soluble): *Streptococcus pneumoniae* ATCC® 49619.

4.3.4.7 Negative control (bile insoluble): *Enterococcus faecalis* (ATCC29212) or *Streptococcus mitis* NCTC 10712.

4.3.4.1 Record quality control result on the quality control sheet (see Annexes #1, Daily microbiology identification test quality control sheet).

4.4. Analytical stage:

There are two methods for this test called plate method and Tube method.

4.4.1 Plate Method (Spot test):

4.4.1.1 Select a well-isolated single colony from a blood agar or chocolate plate. Circle the colony on the bottom of the Petri dish. This will help locate it after testing.

4.4.1.2 Place one drop of 10% sodium deoxycholate directly on the colony. Incubate at 37°C for up to 30 minutes. Do not invert the plate. The lid may be left slightly ajar to aid evaporation.

4.4.1.3 When the reagent has dried examine the area for lysis or disintegration of the original colony (see Fig. No.1).

4.4.1.4 Positive result: colony lysed or disintegrated.

4.4.1.5 Negative result: no change.

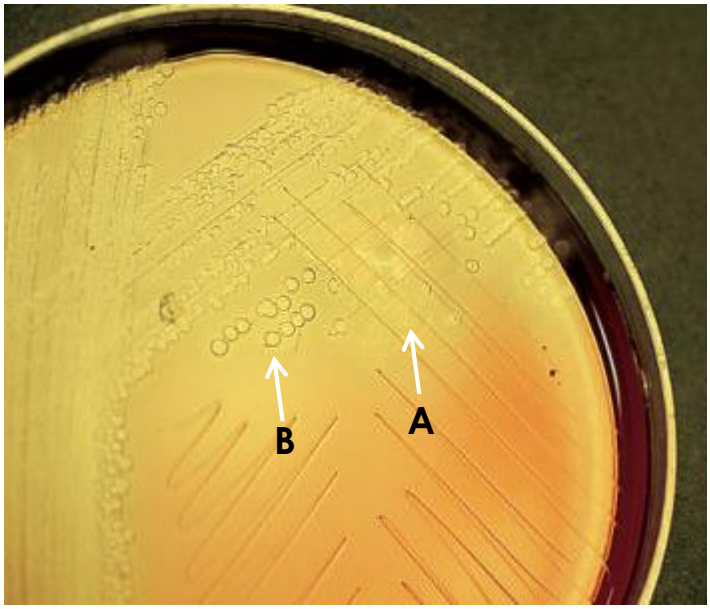

Fig. No.1 Bile solubility test – Plate method:
On 5% sheep blood agar plate (A- Colony Lysed, B- Intact Colony).

4.4.2 Tube Method

4.4.2.1 Prepare a suspension (turbidity 0.5-1 McFarland standard) of a pure culture (controls & test) in 2 ml of 0.85% saline.

4.4.2.2 Divide the organism suspension into two tubes.

4.4.2.3 To one tube (test tube), add 2 drops of 2% sodium desoxycholate and mix.

4.4.2.4 To the other tube (control tube), add 2 drops of sterile distilled water and mix.

4.4.2.5 Leave both tubes for 10-15 minutes at 35-37°C in 5-10% CO_2.

4.4.2.6 Observe for a clearing of turbidity in the tube containing 2% sodium deoxycholate.

4.4.2.7 If negative, continue to incubate up to 3 hours. Observe again for clearing.

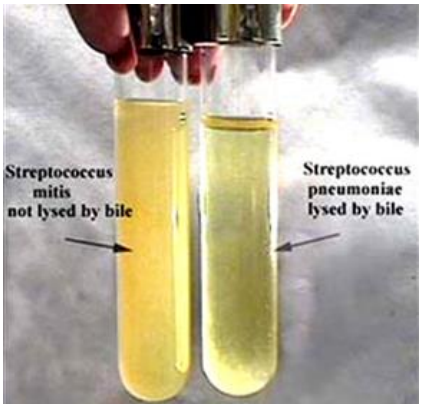

Fig. No.2 Bile solubility test – Tube method.

4.5. Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

4.5.1.1 Plate Method:

- Positive Result: Colony disintegration or flattening of the colony within 30 minutes, leaving an alpha-hemolytic where colony may be located.
- Negative Result: No change i.e. colonies remain intact.

4.5.1.2 Tube Method:

- Positive Result: Suspension clears in tube labelled test and remains turbid in control tube.
- Negative Result: Suspension remains turbid.
- Note: Partial clearing (partial solubility) is not considered positive for *S. pneumoniae identification.*

4.5.2. Limitation of Bile Solubility Test:

- Bile salts will not induce clearing of a killed culture or one that is too acid. Therefore saline suspensions of young cultures are used.
- The test should not be performed on old cultures, as the active enzyme may be lost.
- Normal autolysis of *S. pneumoniae* may be inhibited by a high concentration of bile salts being used. Evaporation may cause the reagent to become more concentrated, therefore affecting the test.
- When performing the bile solubility tube test using saline or unbuffered broth, it is essential to adjust the pH to neutral before adding the reagent in order to avoid false negative reactions.
- When testing using the plate method, care must be taken not to dislodge the colony being tested, therefore leading to false positive results.

5. Responsibilities:

5.1.Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.2.All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended.
- To report test failures or incident.

6. Document History and Version Control:

Version	Description	Review Date
1	Initial Release	May 2026

7. References:

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Diagnostic Microbiology	Bailey & Scott's Diagnostic Microbiology	2007, 13th ed.	231
District Laboratory Practice in Tropical Countries, Part 2	Monica Cheesbrough	2006, 2nd ed.	63-64
Manual of Clinical Microbiology	Patrick R. Murray	2003, 8th Volume 1	411-412
Bile Solubility Test. UK Standards for Microbiology Investigations.	Issued by the Standards Unit, National Infection Service, PHE	2018	
Bile Solubility Reagents, kit insert	Remel	2012	
Bile Solubility Reagent, kit insert	Dalynn Biologicals	2014	

8. Annexes #1: Daily microbiology identification test quality control sheet:

Test Name: Bile solubility test			**Kit Manufacture Name:**
Reagent Lot No:		**Reagent Exp. Date:**	**Reagent Open date:**

Month: ……																														
Positive Control result (P)																														
Negative Control result (N)																														
Quality Control (Pass/Failed)																														
Initials																														

Annexes #2: Flowchart of alpha Hemolytic *Streptococcus* Identification: