Ministry of Health

Document Title	Streptococcus grouping by Latex Agglutination Test SOP
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Directorate/Institution	The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH)
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Date	May 2023	Date	June 2023

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Acknowledgment

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Acronyms:

CSF	Cerebral Spinal Fluid
ATCC	American Type Culture Collection
QC	Quality Control
SOP	Standard operating procedure
COSHH	Control of Substances Hazardous to Health
MSDS	Material Safety Data Sheet

1. **Purpose:**

   This procedure provides instruction for identification and grouping of beta-hemolytic *Streptococcus sp*. by latex test.

2. **Scope:**

   This document is applicable to all laboratory technologist staff in Oman.

3. **Definitions:**

   3.1 Chorioamnionitis: As serious inflammation of the two membranes (Chorion-outer membrane & Amnion-inner membrane) surrounding the foetus during pregnancy caused by bacteria (e.g. Group B *streptococcus*).

4. **Details: procedure:**

   4.1.Pre- analytical stage:

   4.1.1. Clinical background (Lancefield Group *Streptococci*):

   4.1.1.1. *Streptococcus pyogenes* (Group A) is a significant human pathogen and causes a wide variety of infections including acute pharyngitis, scarlet fever, erysipelas, streptococcal cellulitis, necrotising fasciitis, toxic shock syndrome, myositis, lymphangitis, impetigo, puerperal fever. Outbreaks of infection most often occur in surgical, burns and obstetric patients.

   4.1.1.2. *Streptococcus agalactiae* (Group B) is a significant human pathogen which can be found in pregnant women (urine and vaginal swab specimen) and leading cause of neonatal septicemia. It is associated with pneumonia in elderly patients, adult urinary tract infection, chorioamnionitis and endometritis, skin and soft tissue infection, osteomyelitis, and meningitis

   4.1.1.3. *Streptococcus* (Group C & group G) are associated with mild and severe human disease.

   4.1.1.4. *Streptococcus* (Group D) is associated with UTI, bacteraemia and endocarditis.

   4.1.1.5. *Streptococcus* (Group F) is associated with abscess formation and purulent disease.

4.1.2. Principle of the test:

4.1.2.1. Isolates from primary culture are identified by colonial appearance, Gram stain, catalase test, and Lancefield grouping. Beta hemolytic colonies on blood agar, gram positive cocci in pairs or chains, catalase negative is used for qualitative detection and identification of the Lancefield group (A, B, C, D, F and G) of *Streptococci*.

4.1.2.2. Streptococci possess group-specific antigens, which can be extracted in soluble form and identified by precipitation reactions with homologous antisera. Group specific antigens are extracted from streptococci in a simple incubation step. Antigens are then identified using polystyrene latex particles, which have been coated with group-specific antibodies. The latex particles agglutinate strongly in the presence of homologous antigen, and remain in smooth suspension in the absence of homologous antigen.

4.1.3. Note:

4.1.3.1. Non-groupable streptococcus from clinical specimen especially from sterile body sites (e.g. CSF, tissue, body fluids etc.) shall be further tested by other tests such as Bile-Esculin, PYR-Hydrolysis, growth in 6.5% NaCl, resistance to penicillin or Phoenix identification or MALDI-TOF.

4.1.3.2. If further test is not available sent isolate to reference lab.

4.1.4. Sample:

4.1.4.1 Sample type: well young growth of isolated colonies.

4.1.4.2 Sample source: solid culture medium.

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

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

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

4.1.5. Materials:

Reagents	Consumables/Supplies	Equipment
Strept. Latex agglutination kit	• All consumables are included with kit. • Test tube	• Incubator 37c • Fridge (Storage: 2-8°C). • Water bath or a beaker of water.

4.1.6. Safety Precautions:

4.1.6.1 Refer to risk assessment, appropriate COSHH and MSDS documents.

4.1.6.2 Work with standard lab safety practice.

4.1.7. Quality Control:

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

4.1.7.2 Use known positive and negative control internal controls and ATCC strains.

4.1.7.3 Positive control: Polyvalent positive control.

4.1.7.4 Negative control: water or extraction enzyme.

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

4.2.Analytical stage:

4.2.1. Follow package insert instruction to perform the tests as some kit may not require incubation for 10 minutes (such as Streptex Rapid latex kit).

4.2.2. Stepwise instructions for performing the examination (Use operator' Inserts).

4.2.3. Reconstitute the bottle of extraction enzyme with 11 ml of sterile distilled water.

4.2.4. Allow standing for a few minutes (according to manufacturer instruction) with occasional mixing.

4.2.5. Pipette 0.4ml of extraction enzyme into a labeled test tube.

4.2.6. Pick as few as 5 colonies to make a light suspension in the tube.

4.2.7. Incubate at 37°C in a water bath or a beaker of water in an incubator for a minimum of 10 minutes to one hour. Shake the tube after 5 minutes.

4.2.8. Vigorously shake each latex suspension and, holding the dropper bottle vertically dispense one drop of each latex solution onto a separate circle on a reaction card.

4.2.9. Using a pipette, place one drop of incubated extract into each of the six circles on the reaction card.

4.2.10. Mix the contents in each circle with a separate wooden stick, and spread contents over the whole circle.

4.2.11. Rock the card gently for a maximum of 1 minute. Observe and record agglutination pattern.

4.2.12. Discard the card and sticks to the disposable plastic container and then into yellow biohazard bag.

4.2.13. Expected results:

4.2.13.1 A positive result is indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles.

4.2.13.2 A negative result shows no agglutination and the milky appearance remains substantially unchanged throughout the one-minute test.

4.2.14. Limitation of the test:

4.2.14.1 As a general rule the kit only gives reliable results when used to test beta-haemolytic streptococci. The exception to this rule is strains of non-haemolytic streptococcus that group as B or D.

4.2.14.2 Some Group D Streptococci are often difficult to detect using this method, particularly *Enterococcus faecium*, as few antigens may be present. A heavier inoculum or incubation in extraction enzyme for longer periods of time may help. Other tests are such as esculin, PYR, resistance to penicillin or Phoenix identification maybe helpful. Any suspect Enterococci from clinical significant sites should not be reported as non-groupable streptococci until further investigations have been performed.

4.2.14.3 False negative results can occur if the inoculum is too light.

4.2.14.4 False positive results can occur with *Klebsiella sp*., *Escherichia sp*., and, *Pseudomonas sp*.

4.3.Post – analytical stage:

Record the result of streptex latex grouping in the worksheet and follow up with appropriate test.

5. Responsibility:

5.1 Responsible staff shall:

5.1.1 To ensure the adherence to this procedure.

5.1.2 To perform and record the QC.

5.2 Quality manager /officer:

5.2.1 To follow up the implementation of the procedure.

5.2.2 To monitor regularly QC performance of the test and raise non-conformance with corrective action in case of any QC failure.

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
Identification of Streptococcus species, Enterococcus species and Morphologically Similar Organisms. UK Standards for Microbiology Investigations. https://www.gov.uk/uk-standards-for-microbiologyinvestigations-smi-quality-and-consistency-in-clinical-laboratories	Public Health England	(2014)	ID 4 Issue3.
Manual of Clinical Microbiology	Versalovic J. etc.	2011, 10th ed.	331

8. **Annexes:**

8.1 Daily microbiology identification test quality control sheet

Test Name: Strept latex grouping, Panel														**Kit Manufacture Name:** ……..
Lot No:-														
Expiry Date														
Latex Reagent	**Neg.**	**Pos.**	**Neg.**	**Pos.**	**Neg.**	**Pos.**	**Neg.**	**Pos.**	**Neg.**	**Pos.**	**Neg.**	**Pos.**	**Neg.**	**Pos.**
A														
B														
C														
D														
G														
Initial														

8.2 Annexes: Flowchart of β Hemolytic Streptococcus Identification: