Ministry of Health

Document Title	Coagulase 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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Acknowledgment

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Acronyms:

ATCC	American Type Culture Collection
SOP	Standard operating procedure
WHO	World Health Organization

1. **Purpose**

This procedure provides instruction for coagulase test.

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 Coagulase: is a protein enzyme which enables conversion of fibrinogen to fibrin clot of blood plasma. It is found in some microorganism such as *Staphylococcus aureus*, it helps the bacteria to evade detection and phagocytosis by the immune system.

4. **Procedure**

4.1. Clinical background:

The coagulase test is simple, reliable method of identification of *Staphylococcus aureus*. There are two methods for coagulase test slide and tube method. Slide and tube method measures different reactions which explain why some *Staphylococcus* gives negative slide test but positive tube method. In clinical microbiology a positive slide test speeds up the identification of the organism but negative reactions does not eliminate *Staph. aureus* from consideration. There are few factors which affect coagulase test such as the species of organism donating plasma, the age of the inocula, the presence of anticoagulants, and the amount of salt.

4.2. Principle:

Many microorganisms produce coagulase, which converts fibrinogen to fibrin. Coagulase is categorized into two forms: (bound coagulase) or (clumping factor) which is bound to the cell wall and (free coagulase) which is liberated by the cell wall. With the slide coagulase test, bound coagulase can be detected whereas both free and bound coagulase can be detected with the tube coagulase test.

Tube coagulase tests reveal the presence of "free" coagulase, an intra-cellular enzyme produced by *Staphylococcus, coagulase positive*. Prothrombin is reduced by free coagulase to form thrombin-like product, which, in turn, is converted by fibrinogen to form a fibrin clot.

4.2.1 Note:

4.2.1.1 To keep expense at minimum it is possible to run control once a day.

4.2.1.2 As this test is primary used to differentiate between *Staphylococcus sp*. it can be used as well to differentiate other clinical important similar organism. *Peptostreptococcus* indolicus is positive while other *Peptostreptococcus* species are negative.

Erysipelothrix rhusiopathiae is positive

while *Listeria* and *Corynebacterium* spp. are negative.

4.3.Pre – analytical stage:

4.3.1.Sample:

4.3.1.1 Sample type: fresh well isolated colonies.

4.3.1.2 Sample source: Solid or liquid media.

4.3.1.3 Amount of sample required: Colonies from non inhibitory agar medium.

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

4.3.1.5 Criteria for unacceptable samples and follow-up action: Mix growth or not well isolated colonies are unacceptable for coagulase test and therefore subculture on purity plate.

4.3.2.Material:

Reagents	Consumables/Supplies	Equipment
➢ Lyophilized Rabbit plasma. ➢ If reconstituted stable for: 5 days at 2-8°C. 30 days at -20°C. ➢ Not exceeding expiry date on the label.	➢ Transfer pipette. ➢ Test tubes. ➢ Glass slides. ➢ Wooden applicator. ➢ Bacteriological Disposable wire/loop. ➢ Non inhibitory agar medium.	➢ Incubator. ➢ Fridge.

4.3.3.Safety precaution:

4.3.3.1 All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times.

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

4.3.3.3 Lyophilized Rabbit plasma is not considered as hazardous.

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

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:

Use known positive and negative control with known ATCC strains.

- ✓ Positive Control: ATCC *Staphylococcus aureus* ATCC @ 29213.
- ✓ Negative Control: *Staphylococcus epidermidis* ATCC @ 12228.

4.3.4.4 Record the quality control results in the appropriate QC sheet (see Annex 1, Daily microbiology identification test quality control sheet).

4.3.4.5 Follow the slide / tube method for the controls using the same procedure as the test.

4.3.4.6 Proceed for patient isolate if the QC passed.

4.4. Analytical stage:

- Reconstitution instruction of rabbit plasma shall be followed as per manufacture instruction.
- Storage condition of the rabbit plasma shall be followed as per manufacture recommendation.
- There two methods for coagulase test, they are: Slide coagulase test and Tube coagulase test.

4.4.1 Slide coagulase test direct from colonies (Bound coagulase):

- Perform slide coagulase test with the control first before using the plasma for patient isolates**.**
- If the QC passed, then proceed for the patient's isolate as follows:

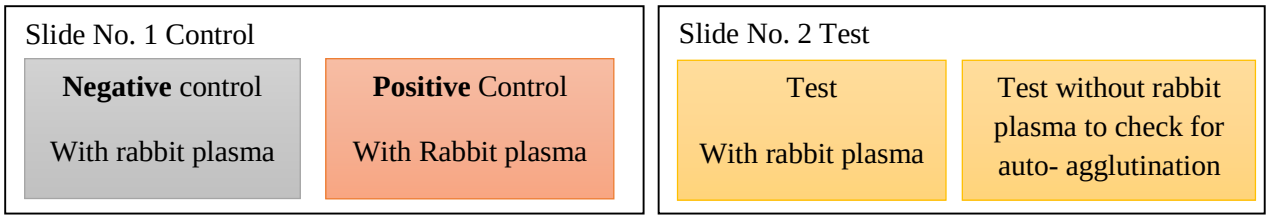
4.4.1.1 Label glass slide with test / control.

4.4.1.2 Place one drop of sterile distilled water or saline on the end of the glass slide.

4.4.1.3 Emulsify a portion of the colony with loop, straight wire or wood stick in the drop to make thick suspension.

4.4.1.4 Add one drop of rabbit plasma to the suspension on slide and mix gently.

4.4.1.5 Within 10 seconds observe for visible clump on the slide.



4.4.2 Tube coagulase test direct from colonies:

- Perform tube coagulase test together with the control in parallel with patient isolate.
- If the QC passed, then proceed for the patient's isolate as follow:

4.4.2.1 Follow manufacturer recommendation for plasma reconstitution, dilution and storage condition.

4.4.2.2 Label 3 test tubes with (T) test, (N) negative control, and (P) positive control.

4.4.2.3 Add 0.5 ml of plasma into each tube.

4.4.2.4 Emulsify 2-4 colonies into plasma.

4.4.2.5 Mix and incubate at 36°C ±1 for 4 hours.

4.4.2.6 Examine the tube for agglutination within 4 hours.

4.4.2.7 If no visible clot was observed, leave at room temperature overnight and examine in the morning.

4.4.2.8 Do not shake or agitate the tube, check for clot.

4.4.2.9 Record the test /control results in the appropriate sheet.

4.4.3 Tube coagulase test direct from blood culture broth:

- The direct coagulase tube test from blood culture broth can be used to early detection of *Staphylococcus* Bacteriamia, reduce turnaround time and initiate appropriate antimicrobial agent.
- Follow the bellow procedure:

4.4.3.1 Add 0.1 ml of blood culture to 0.4 ml of rabbit plasma.

4.4.3.2 Mix and incubate at 36°C ±1 for 4 hours.

4.4.3.3 Examine the tube for agglutination within 4 hours.

4.4.3.4 If no visible clot was observed, leave at room temperature overnight and examine in the morning.

4.4.3.5 Do not shake or agitate the tube, check for clot.

4.5.Post – analytical stage:

4.5.1.Interpretation / Results / Alerts:

4.5.1.1 Positive result: Formation of a clot up to 4hr at 37°C or following overnight incubation at room temperature (22°C).

4.5.1.2 **Negative result:** No clot, plasma moves freely at 4hr and 24hr incubation.

4.5.1.3 Table No.1 Summary of some Staphylococcus species that may cause infections in humans with expected coagulase test result:

Species	Tube coagulase test	Slide coagulase test
Staphylococcus aureus subspecies aureus	+	+
Staphylococcus aureus subspecies anaerobius	+	-
Staphylococcus epidermidis	-	-
Staphylococcus haemolyticus	-	-
Staphylococcus saprophyticus subspecies saprophyticus	-	-
Staphylococcus schleiferi subspecies coagulans	-	+
Staphylococcus lugdunensis	-	+
Staphylococcus schleiferi subspecies schleiferi	v	+
*Staphylococcus delphini**	+	-
*Staphylococcus intermedius**	+	v
*Staphylococcus hyicus**	V	-
V= variable reaction +/- **rare clinical isolates*	*- = negative reaction* *+=positive reaction*	

4.5.2.Reporting:

Coagulase test:

- Positive: suggestive of *Staphylococcus aureus*.
- Negative: suggestive of *Staphylococcus,* Coagulase Negative.

4.5.3.Limitation:

4.5.3.1 A flocculent or fibrous precipitate is not true clot and should be recorded as negative.

4.5.3.2 False negative result may occur for some strains producing staphylokinase which may lyse the clot after formation (after long incubation).

4.5.3.3 False positive result may occur due auto-agglutination, colonies from media containing high concentrations of salt (e.g. Mannitol Salt agar) or from using Saline.

5. Responsibilities:

5.1 Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2 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.

5.3 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
Evaluation of direct tube coagulase test in diagnosing staphylococcal bacteremia. J Clin Diagn Res. 2014;8(5):DC19-DC21. doi:10.7860/JCDR/2014/6687.4371.	Thirunavukkarasu S, K C R.	2014	
Error! Reference source not found.. UK Standards for Microbiology Investigations. TP **Error! Reference source not found.** Issue **Error! Reference source not found.**. (https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories).	Public Health England	2018	
Coagulase Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 105-19. B, III.	MacFaddin JF.	2000	
Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). JAntimicrobChemother 2005;56:1000-18. A, IV.	Brown DF, Edwards Di, Hawkey PM, Morrison D, Ridgway GL, Towner KJ et al.	2005	
Manual of Clinical Microbiology, 10th ed., vol. 2.	Versalovic J., Carrol K. C., Funke G., Jorgenesn J. H., Landry M. L., and Warnock D. W.	2011	
Coagulase Test Protocol, American Society for Microbiology, visited web site on 07-06-2022 (https://www.asmscience.org/docserver/ful	Katz S.	2010	

ltext/education/protocol/protocol.3220.pdf?expires=1623052062&id=id&accname=guest&checksum=D53A27C6DA36F5CDB100AD0764E9F9D1).			

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

Test Name: Coagulase 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																														