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Coagulase Reagent

Lyophilized rabbit plasma is used for the qualitative detection of the coagulase enzyme produced by *Staphylococcus aureus*

REF: BS.1/CG01.005.0005	5 Test	REF: BS.1/CG01.010.0010	10 Test
REF: BS.1/CG01.030.0030	30 Test	REF: BS.1/CG01.050.0050	50 Test

CLINICAL SIGNIFICANCE

Differentiation of Staphylococcus aureus from the coagulase negative species, including Staphylococcus epidermidis and Staphylococcus saprophyticus, is critical not only because Staphylococcus is a health risk of major importance but also because the latter species are progressively associated with septicaemia, bacterial endocarditis, colonization of prostheses and infections of the urinary tract. . Identification of staphylococci is based on the morphology of the colony, cultural and biochemical characteristics and microscopic examination. Nevertheless, the detection of coagulase is the most widely used criterion for differentiation between species.

METHOD PRINCIPLE

Coagulase acts on a constituent of rabbit plasma (coagulase reacting factor) to produce a thrombin-like substance. This substance activates fibrinogen to form fibrin which results in the formation of a fibrin clot. Coagulase is present in two forms:
bound coagulase or clumping factor remains attached to the cell wall of the organism which is to be detected; free coagulase is an extracellular enzyme produced when the organism is cultured in broth.

REAGENT COMPOSITION

Coagulase Plasma is lyophilized rabbit plasma to which EDTA is added as an anti-coagulant. EDTA is not consumed by bacteria, thus will not cause false positive coagulase reactions by bacteria that can utilize citrate.

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Coagulase Reagent material safety data sheet.

REAGENT PREPARATION

Rehydrate lyophilized Coagulase Plasma with 0.5ml sterile distilled water

STORAGE, STABILITY

BioScien Coagulase Reagent is stable until expiration date stated on label when properly stored at 2-8°C for non-reconstituted and unopened vials. Aliquots may be tightly capped and frozen at -20°C or below for up to 1 month or refrigerate at 2-8°C for 5 days. Frozen aliquots of Coagulase Plasma should not be refrozen after thawing.

Procedure

A. Tube Coagulase test

1. Using a sterile 1 ml pipette, add 0.5 ml of the reconstituted plasma to a test tube.
2. Using a sterile loop emulsify 2-4 colonies of the test organism into the plasma.
3. Mix gently and DON'T SHAKE.
4. Incubate for 4 hours in a water bath at 37°C.
5. After 1 hour check for clot formation by gently tipping the tube onto its side. If no clot formation is evident examine every 30 minutes up to the 4 hour limit.
6. If there is no clot after 4 hours incubation, reincubate the test at room temperature for the remaining time period and examine for clot formation at 24 hours. Do not reincubate tests that have already produced a clot at 4 hours as some strains of *S. aureus* will produce a fibrinolysin that may lyse the clots after further incubation.
7. Record the results.

B. Slide Coagulase Test

(For detection of Bound coagulase or Clumping Factor)
1. Place a drop of the reconstituted plasma and a drop of saline side by side on a clean, dry glass slide.
2. Emulsify a loopful of the colonies to be tested into the drop of plasma and into the saline.
3. Observe for visible clumps for up to one minute.
4. Record results.

Deterioration

The color of ***BioScien*** Coagulase Reagent should not be used if the plasma is clotted upon rehydration, the product is contaminated, the expiration has passed, or there is additional sign of deterioration.

SPECIMEN PREPARATION

The test isolate should be pure and has the following characteristics of Staphylococcus aureus:
• Appropriate morphology on the isolation medium.
• Gram positive-cocci
• Catalase positive.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile distilled water
- Inoculating loops
- test tubes
- Sterile 1 ml pipettes
- Water bath (37°C)
- Glass slides
- Positive & Negative Control Strains (Refer to Quality Control).

RESULT INTERPRETATION

A. Tube Coagulase Test
• Positive Result: Any degree of clotting of the coagulase plasma observed within 24 hours.
• Negative Result: No clotting of the coagulase plasma**.**
B. Slide Coagulase Test
• Positive Result: Macroscopic clumping in the plasma within one minute and no clumping in the saline.
• Negative Result: No clumping in both the plasma and the saline.
• Uninterruptable Result: If clumping is seen in both tests, it indicates that the isolate has auto agglutinated and is unsuitable for the slide agglutination test. If this is observed the isolate should be tested using the tube coagulase test.

QUALITY CONTROL

Organism	Expected Result Test Tube	Expected Result Slide Test
Staphylococcus aureus (ATCC 25923)	Clot formation	Clump Formation
Staphylococcus epidermidis (ATCC 12228)	No clot formation	No clump formation

REFERENCES

1. Bannerman, T.L. and Peacock, S.J. (2007). Staphylococcus, Micrococcus,
and Other Catalase-Positive Cocci, In Manual of Clinical Microbiology, 9th Edition. Edited by Murray, P.R., Baron, E.J., Landry, M.L., Jorgensen, J.H. and
Pfaller, M.A. American Society for Microbiology, Washington, D.C. page 390-411.
2. Loeb, L. (1903). The influence of certain bacteria on the coagulation of the blood. J. Med. Res. 10:407-419.
3. Chapman, G.H., Berens, C., Nilson, E.L. and Curcio, L.G. (1938). The differentiation of pathogenic Staphylococci from non-pathogenic types. J. Bact. 35:311-333.
4. Morton, H.E. and Cohn, J. (1972). Coagulase and deoxyribonuclease activities of Staphylococci isolated from clinical sources. Applied Micro. 23-725-733.
5. Baird-Parker, A.C. (1974). Staphylococcus In Bergey's Manual of Determinative Bacteriology, 8th Edition. Edited by Williams and Wilkins. Baltimore. Page 484-489.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		