



TCS Biosciences Ltd
Botolph Claydon
Buckingham, MK18 2LR
United Kingdom
Telephone: +44 (0) 1296 714222
Fax: +44 (0) 1296 714806
E-mail: sales@tcsgroup.co.uk

RABBIT COAGULASE PLASMA – CP6X3

Sold lyophilised as a pack of 6 x 3ml vials

Intended Use

Rabbit Coagulase Plasma is a standardised, lyophilised rabbit plasma used for detecting coagulase enzyme produced by *Staphylococcus aureus*

Summary and Explanation of the Tests

Coagulase Detection

Identification of staphylococci is based on microscopic examination, colonial morphology, cultural and biochemical characteristics and the ability to clot plasma by the action of the enzyme coagulase.

Principles of the Procedure

Staphylococcus aureus produces two types of coagulase, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as clumping factor, remains attached to the cell wall of the organism.

The slide test is performed by adding a heavy suspension of cells to a drop of plasma on a slide, then observing for the presence or absence of agglutination. This test only detects bound coagulase and is used infrequently because it is less accurate, is subject to time related false-negative results and requires that negative results be confirmed by the tube test.

The tube test consists of adding *S. aureus* from an overnight broth culture or from a non-inhibitory agar plate to a tube of rehydrated coagulase plasma and then incubating at 37°C. The formation of a clot in the plasma indicates coagulase production. The tube test is the most frequently used method because of its greater accuracy and its ability to detect both bound and free coagulase.

Reagents

Coagulase Plasma is lyophilised rabbit plasma to which EDTA has been added as the anticoagulant. EDTA is not utilised by bacteria and therefore will not give rise to false-positive coagulase reactions by bacteria which utilise citrate.

Rehydration

Rehydrate Coagulase Plasma by adding sterile distilled or deionised water to the vial, as indicated on the label. Mix by gentle rotation of the vial. If, upon rehydration, the plasma is not in complete solution or if fibrin clots or strands are evident, discard the plasma and test the pH of the distilled water. An acid pH of the water could result in an unsatisfactory reagent.

Storage

Store unopened Coagulase Plasma at 2-8°C.

Store reconstituted plasma at 2-8°C or aliquot in 0.5ml quantities, freeze promptly and store at -20°C. Do not thaw and refreeze.

Expiration Date

Unopened Coagulase Plasma is stable until the expiration date on the label when stored as directed.

Reconstituted plasma, if kept uncontaminated, retains its activity for five days when stored at 2-8°C or for up to 30 days when aliquoted and stored at -20°C, not exceeding the expiration date on the label.

Specimen Preparation

Coagulase Detection

1. Determine that the test culture is pure and has the characteristics of *Staphylococcus aureus*.

Gram Stain:	Gram-positive cocci in grape-like clusters
Catalase test:	Positive

2. Using a bacteriological loop, transfer a well-isolated colony from a pure culture into a tube of sterile Brain Heart Infusion broth. Incubate at 37°C for 18-24 hours or until dense growth is observed. Alternatively, take 2-4 colonies (one loopful) directly from a pure culture on a non-inhibitory agar plate as an inoculum instead of the broth culture.

Tube Coagulase Test

1. Using a sterile 1ml pipette, add 0.5ml of the rehydrated plasma to a 12x75mm test tube.
2. Using a sterile 1ml serological pipette, add two drops of the overnight broth culture of the test organism to the tube of plasma or, using a sterile bacteriological loop, thoroughly emulsify 2-4 colonies (one loopful) from a non-inhibitory agar plate in the tube of plasma.
3. Mix gently and incubate in a water bath at 37°C for 4-24 hours. If it is necessary to use an incubator, it must be one without a CO₂ atmosphere since the presence of CO₂ may cause false-positive results.
4. Examine periodically for coagulation by gently tipping the tube after the first hour and once every hour thereafter until four hours have elapsed. If desired, reincubate and examine after 24 hours. Avoid shaking or agitating the tube during reading. Doubtful or false-negative results may occur due to breakdown of the clot. Record results.

Results

Coagulase Production.

Any degree of clotting of the Coagulase Plasma in up to 24 hours constitutes a positive test.

Quality Control

Use known positive and negative control cultures in parallel with the test culture to ascertain the validity of test results.

Organism	Expected Results
<i>Staphylococcus aureus</i> NCTC 12981/ATCC® 25923	Clot in COAGULASE TEST
<i>Staphylococcus epidermidis</i> NCTC 13360/ATCC® 12228	No clot in COAGULASE TEST

Limitations

1. The slide agglutination technique for determining the coagulase activity of staphylococci is not recommended since false-positive and false negative reactions may occur.
2. When checking results of the COAGULASE TEST, tubes should be observed hourly during the first four hours of incubation. Some strains of *S. aureus* produce fibrinolysin which may lyse clots formed earlier. If the tubes are not read until 24 hours of incubation, reversion to a false negative might result.