PRO-LAB DIAGNOSTICS

RABBIT COAGULASE PLASMA

FOR IN VITRO DIAGNOSTIC USE

PRODUCT CODE PL850, PL850-5, PL850-10, PL850-20, PL850-30

CE

INTENDED USE.

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

SUMMARY AND EXPLANATION OF THE TESTS.

Differentiation of *Staphylococcus aureus* from the coagulase negative species, including *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, is crucial not only because *Staphylococcus* is a health risk of prime importance but also because the latter species are increasingly associated with septicaemia, bacterial endocarditis, colonization of prostheses and infections of the urinary tract. Identification of Staphylococci is based on colonial morphology, cultural and biochemical characteristics and microscopic examination. However, the detection of coagulase is the most widely used criterion for differentiation between species. The ability of *Staphylococcus* to produce coagulase, an enzyme capable of clotting plasma, was first reported by Loeb in 1903. Dorang indicated the practical significance of this test. Since that time, many investigators have tried to correlate the production of coagulase with the pathogenicity of Staphylococci. Chapman, Berens, Peters and Curcio, in a study of coagulase and haemolysin production by *Staphylococcus*, showed that strains producing coagulase were usually pathogenic, regardless of their haemolytic or chromogenic properties.

More recent experience has demonstrated that the ability of a staphylococci to produce coagulase cannot be relied upon to always indicate its pathogenicity.

PRINCIPLES OF THE PROCEDURE.

Staphylococcus aureus produces two types of coagulase, free and bound. Free coagulase is an extra cellular 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 tube test consists of adding *S. aureus* from overnight broth culture or from a non inhibitory agar plate to a tube of rehydrated coagulase plasma and 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. The slide test is performed by making a heavy suspension of the test organism in a drop of saline on a clean glass slide, a drop of plasma is then added and observed for the presence of a clot. This test is less accurate, and subject to time related false negative results, and requires that negative results be confirmed by the tube test. The slide test is only capable of detecting bound coagulase.

REAGENTS.

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

REHYDRATION.

Rehydrate Coagulase Plasma by adding sterile distilled or deionized water to the vial, the volume to be added is as indicated on the vial. Mix by gentle rotation of the vial insuring complete dissolving. If, upon rehy dration, 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 vials at 2-8°C. Store reconstituted plasma at 2-8°C, or aliquot into 0.5 ml volumes, freeze promptly and store at -20°C. Do not thaw and refreeze.

EXPIRATION DATE.

Unopened Coagulase Plasma vials are stable until the expiration date shown on the product label when stored as directed. Reconstituted Coagulase Plasma, if kept uncontaminated, will retain 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 expiry date on the label.

SPECIMEN PREPARATION.

Coagulase Detection.

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

   Morphology (Medium dependent)

   Blood Agar Base with 5% horse blood- Opaque, yellow to orange, with haemolysis.

   Coagulase Mannitol Agar - Opaque with yellow to orange zones.

   DNase Test Agar with Methyl Green- Clearing of green dye.

   Mannitol Salt Agar - Yellow to orange surrounded by yellow zones.

   Staphylococcus Medium 110 - Yellow to orange.

   Tellurite Glycine Agar - Black.

   VJ Agar - Black, surrounded by yellow zones.

   Baird Parker Agar - Grey to Black shiny colonies, surrounded by zones of clearing.

   Gram Stain.

   Gram positive cocci appearing in grape like clusters or, occasionally, in chains.

   Catalase Test. Positive.

   Mannitol Fermentation. Positive.

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

COAGULASE TEST.

1. Using a sterile 1 ml pipette, add 0.5 ml of the rehydrated plasma to a 12 x 75 ml test tube.
2. Using a sterile 1 ml pipette, add two drops of the overnight broth culture of the test organism to the tube or, using a sterile bacteriological loop, thoroughly emulsify 2-4 colonies (one loop full) from a non-inhibitory agar plate, in the tube of plasma.
3. Mix gently.
4. Incubate in a water bath at 37°C for 4-24 hours. If it is necessary to use an incubator, it must be without a CO_2 atmosphere since the presence of CO_2 may cause false-positive results.
5. Examine periodically for coagulation by gently tipping the tube after the first hour and once every hour thereafter until four hours have elapsed. If necessary, 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.
6. Record results.

INTERPRETATION OF RESULTS.

Any degree of clotting observed of the coagulase plasma within 24 hours constitutes a positive test.

QUALITY CONTROL.

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

Organism		Expected Result
S. aureus	ATCC 25923	Clot Formation
S. epidermidis	ATCC 12228	No Clot Formation

LIMITATIONS.

1. When using the slide agglutination method for determining coagulase activity in Staphylococci, false positive reactions may occur with some strains when animal plasmas are used. In addition, spontaneous agglutination may occur when rough cultures are used. When the slide method is employed, all negative reactions should be confirmed by the tube test.
2. Some species or organisms utilize citrate in their metabolism and may yield false-positive reactions for coagulase activity. Normally this would not cause problems since the coagulase test is performed on Staphylococci almost exclusively. However, it is possible for bacteria which utilize citrate to contaminate *Staphylococcus* cultures on which the coagulase test is being performed and they may, upon prolonged incuba tion, give false-positive results due to utilization of the citrate. The pres ence of EDTA in the coagulase plasma should overcome this problem.

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3. When checking results of the Coagulase Test, tubes should be observed hourly during the first four hours of incubation. Some strains of *Staphylococcus 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 may occur.

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= Use by

LOT = Lot number

REF = Catalogue number


= Manufacturer

IVD = in vitro diagnostic medical device.


= Temperature limitation


= Consult instructions for use.