

INTENDED USE

Rabbit Coagulase Plasma is standardised lyophilised rabbit plasma used for the qualitative detection of the coagulase enzyme produced by *Staphylococcus aureus*.

SUMMARY AND EXPLANATION

Differentiation of *Staphylococcus aureus* from 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, colonisation of prostheses and infections of the urinary tract. Identification of *Staphylococcus* 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. Since that time, many investigators have tried to correlate the production of coagulase with the pathogenicity of *Staphylococci*. In a study of *Staphylococcus* coagulase and haemolysin production, Chapman, Berens, Nilson and Curcio showed that strains producing coagulase were usually pathogenic regardless of their haemolytic or chromogenic properties. However, more recent experiments have demonstrated that the ability of *Staphylococci* to produce coagulase cannot always be relied upon to indicate its pathogenicity.

PRINCIPLE OF THE TEST

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 tube test is performed by adding 2-4 colonies of the isolate to a tube containing the rehydrated coagulase plasma and incubating at 37°C for up to four hours. The formation of a clot indicates coagulase production. The tube test is the most frequently used method because of its greater accuracy due to its ability to detect both bound and free coagulase.

The slide test is performed by making a heavy suspension of the test isolate in a drop of plasma. This is mixed gently with a loop and examined for clumping. This test is less accurate than the tube test and requires that all negative tests be confirmed by the tube test. This test only detects bound coagulase.

Coagulase Plasma is lyophilised rabbit plasma to which EDTA is added as the anticoagulant. EDTA is not utilised by bacteria, thus will not cause false positive coagulase reactions by bacteria that utilise citrate.

MATERIALS PROVIDED

- PL.850-3 10 x 3ml vials / box
- PL.850-10 10ml vial
- PL.850-20 20ml vial
- PL.850-30 30ml vial
- Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Sterile distilled or deionised water
- Inoculating loops
- Test tubes
- Sterile plastic pipettes
- Water bath / incubator (37°C)
- Glass slides

STABILITY AND STORAGE

- Store unopened, non-reconstituted vials at 2°C-8°C.
- Unopened, non-reconstituted vials will remain stable until the expiration date on the product label if stored as directed.
- Store the reconstituted vials of plasma at 2°C-8°C, or aliquot into 0.5ml volumes, freeze promptly and store at -20°C. Do not thaw and refreeze.
- The reconstituted plasma will remain stable for five days if stored at 2°C-8°C or for up to 30 days when aliquoted and stored at -20°C, not exceeding the expiry date on the label.

PRECAUTIONS

- Rabbit Coagulase Plasma is intended for *in vitro* diagnostic use only.
- Do not use the reagent after the expiry date shown on the product label.
- Universal precautions should be taken in handling, processing and discarding all clinical specimens and reagents.
- The reagent contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
- The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.

SAMPLE STORAGE AND COLLECTION

Coagulase Reconstitution

Reconstitute the Rabbit Coagulase Plasma by adding sterile distilled or deionised water to the vial in the volume indicated on the product label. Gently rotate the vial until the product has completely dissolved. If the product does not completely dissolve or contains fibrin clots or strands do not use the product.

Specimen Preparation

Determine that the test isolate is pure and has the following characteristics of *Staphylococcus aureus*: appropriate morphology on the isolation medium, Gram-positive cocci, catalase-positive. Using a bacterial 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, colonies can be taken directly from a pure culture on a non-inhibitory agar plate as an inoculum instead of the broth culture.

TEST PROCEDURE

A. Tube Coagulase Test

(Detects both bound coagulase and free coagulase)

1. Using a sterile 1ml pipette, add 0.5ml of the reconstituted Rabbit Coagulase Plasma to a test tube.
2. Using a sterile 1ml pipette, add two drops of the overnight broth culture (approximately 100ul) of the test organism to the plasma, or using a sterile loop emulsify 2-4 colonies of the test organism into the plasma.
3. Mix gently.
4. Incubate for 4 hours in a water bath or incubator at 37°C.
5. After 1 hour examine 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 formation after 4 hours incubation, leave the test at room temperature 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 *Staphylococcus aureus* will produce a fibrinolysin that may lyse the clots after further incubation.
7. Record results.

B. Slide Coagulase Test

(Detects bound coagulase)

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 each of the drops.
3. Observe for visible clumps in the plasma for up to one minute.
4. Record results.

QUALITY CONTROL PROCEDURE

Use known positive and negative control cultures in parallel with the test in order to ascertain the validity of test results. A selection of first-generation control material is available; please see the Proclut® range.

Organism	Expected Result Tube Test	Expected Result Slide Test
<i>Staphylococcus aureus</i> (PLD13) NCTC® 12981 / ATCC® 25923*	Clot formation	Clump formation
<i>Staphylococcus aureus</i> (PLD85) NCTC® 6571 / ATCC® 9144*	Clot formation	Clump formation
<i>Staphylococcus epidermidis</i> (PLD15) NCTC® 13360 / ATCC® 12228*	No clot formation	No clump formation

INTERPRETATION OF RESULTS

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.










Invalid Result: If clumping is seen in both tests, it indicates that the isolate has auto-agglutinated and is unsuitable for the slide coagulase test. If this is observed the isolate should be tested using the tube coagulase test.

LIMITATIONS OF THE PROCEDURE

- Although the tube and slide coagulase tests have excellent agreement, false positive slide coagulase tests may occur with other *Staphylococci* species that produce clumping factor. These will include strains of *S. lugdenensis* and *S. schleiferi* subsp. *schleiferi*.
- Some species or organisms utilise 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 utilise citrate to contaminate *Staphylococcus* cultures on which the coagulase test is being performed and they may, upon prolonged incubation, give false positive results due to utilisation of the citrate. The presence of EDTA in the coagulase plasma should overcome this problem.
- When checking results of the coagulase test, tubes should be observed every 30 minutes during the first 4 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.

REFERENCES

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	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use

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