

INTENDED USE

Rabbit Coagulase Plasma is a standardized, lyophilised rabbit plasma used for the qualitative detection of 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 species¹. The ability of *Staphylococcus* to produce coagulase, an enzyme capable of clotting plasma, was first reported by Loeb in 1903². Since that time, many investigators have tried to correlate the production of coagulase with the pathogenicity of staphylococci. Chapman, Berens, Nilson 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 properties³. More recent experience has demonstrated that the ability of staphylococci to produce coagulase cannot be relied upon to always indicate its pathogenicity⁴.

PRINCIPLE 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 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 saline on a clean glass slide and then adding a drop of plasma. This is then 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.

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 citrate⁵.

The Rabbit Coagulase Plasma is supplied in a variety of sizes:

Product Code	Size	Volume for Reconstitution	Approximate # of Tube Tests
PL.850-3	10 vials / box	3 ml / vial	6 x 10
PL.850-5	10 vials / box	5 ml /vial	10 x 10
PL.850-10	Individual vial	10 ml / vial	20
PL.850-20	Individual vial	20 ml / vial	40
PL.850-30	Individual vial	30 ml / vial	60

RECONSTITUTION

Reconstitute the Rabbit Coagulase Plasma by adding sterile distilled or deionized 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.

MATERIALS REQUIRED BUT NOT PROVIDED

Sterile distilled or deionized water
 Inoculating loops
 12 mm x 75 mm test tubes
 Sterile 1 ml pipettes
 Water bath (37°C)
 Glass slides
 Positive & Negative Control Strains (Refer to Quality Control).

PRECAUTIONS

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

STABILITY AND STORAGE

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

1. 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.
2. Use 2-4 colonies (one loop full) of the test organism.

TEST PROCEDURE
A. Tube Coagulase Test

1. Using a sterile 1 ml pipette, add 0.5 ml of the reconstituted plasma to a 12 mm x 75 mm test tube.
2. 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 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 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 results.

B. Slide Coagulase Test

(Detects 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.

QUALITY CONTROL

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

Organism	Expected Result Tube Test	Expected Result Slide Test
<i>Staphylococcus aureus</i> ATCC 25923	Clot formation	Clump formation
<i>Staphylococcus epidermidis</i> 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.
- **Uninterpretable Result:** If clumping is seen in both tests, it indicates that the isolate has autoagglutinated and is unsuitable for the slide agglutination test. If this is observed the isolate should be tested using the tube coagulase test.

LIMITATIONS

1. Although the Tube and Slide coagulase tests have excellent agreement false positive Slide Coagulase tests may occur with other staphylococcal species that produce clumping factor. These will include strains of *S. lugdenensis* and *S. schleiferi* subsp. *schleiferi*.
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 incubation, give false-positive results due to utilization of the citrate. The presence of EDTA in the coagulase plasma should overcome this problem.
3. When checking results of the Coagulase Test, tubes should be observed every 30 minutes 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.



PERFORMANCE CHARACTERISTICS

Pro-Lab Rabbit Coagulase Plasma was evaluated using 30 strains of *S. aureus* and 30 strains of coagulase negative Staphylococci (2 *S. epidermidis*, 4 *S. warneri*, 2 *S. simulans*, 2 *S. hominus*, 2 *S. capitis*, 2 *S. cohnii*, 2 *S. auricularis*, 2 *S. xylo-sus*, 2 *S. sciuri*, 1 *S. oxford*, 3 *S. saprophyticus*, 1 *S. avium* and 5 *S. haemolyticus*). The Pro-Lab Rabbit Coagulase correctly identified all strains indicating the product had a sensitivity of 100% and specificity of 100% in the study.

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.



= Use by

LOT

= Lot number

REF

= Catalogue number



= Manufacturer

EC|REP

= Authorized Representative in the European Community

IVD

= In vitro diagnostic medical device



= Temperature limitation



= Consult instructions for use