



Mast House, Derby Road, Bootle, Merseyside L20 1EA, United Kingdom.
Tel: +44 (0)151 933 7277 Fax: +44 (0)151 944 1332
www.mastgrp.com

MAST® PLASMA COAGULASE

For the detection of coagulase enzyme activity in Staphylococci

The tube coagulase test is a valid means of identifying coagulase positive staphylococci including methicillin resistant strains, provided that only a firm clot (one that does not move when the tube is tipped) is considered a strong positive result.

Identification of staphylococci is based on microscopic examination, colonial morphology, and cultural and biochemical characteristics. Some species of staphylococci, notably *Staphylococcus aureus* in humans and *Staphylococcus intermedius* and *Staphylococcus hyicus* in animals, are associated with acute infections. The organisms can all clot plasma and this ability, due to the presence of the enzyme coagulase, is widely used as a criterion for identification of these pathogens. Coagulase production is very closely linked with the capability of *S. aureus* to form enterotoxins.

Two forms of coagulase exist: one is bound to the cell, called 'bound' coagulase, which acts directly on the fibrinogen in the plasma and causes the bacteria to clump (also known as clumping factor). The other form of coagulase is excreted from the cell as an enzyme, called 'free' coagulase, and this converts prothrombin to a product that then acts on fibrinogen in the plasma to form a fibrin clot.

MAST® coagulase can reliably detect the presence of both bound and free coagulase. It is suitable for both slide and tube coagulase tests.

Some workers prefer to use EDTA plasma rather than citrated plasma, thus avoiding the possibility of false positives with citrate utilising bacteria.

Presentation

Code	Description	Pack size
SV78/3ML	Coagulase Plasma (EDTA)	10 x 3ml
SV78/20ML	Coagulase Plasma (EDTA)	6 x 20ml

Directions

Tube coagulase test:

1. Rehydrate the vial using the diluent specified on the pack label.
2. Shake the vial to ensure the Plasma Coagulase is completely reconstituted.
3. Aliquot 0.5ml reconstituted Plasma Coagulase into suitable sterile tubes using a sterile pipette.
4. Using a culture less than 24 hours old, inoculate the Plasma Coagulase by emulsifying one loopful (2-4 colonies) of bacteria, from a non-inhibitory agar plate, into the tube of plasma.
5. Incubate the inoculated tube at 37°C for 4 hours.
6. Examine for clot formation at hourly intervals.
7. Negative tests at 4 hours should be left at room temperature overnight and examined again for clot formation.

Slide agglutination test:

1. Place a drop of rehydrated Plasma Coagulase onto a clean, dry glass slide.
2. Place a drop of sterile water onto the slide to act as a control slide.
3. Using a sterile loop emulsify an amount of the isolated colony being tested into each drop, inoculating the water first. Try to create a smooth suspension.
4. Observe for clumping on the Plasma Coagulase slide and a homogenous suspension on the control slide.

Interpretation of results

Tube coagulase test:

- Results should be read at 4 hours.
- A positive test for coagulase production results in a clotting of the rabbit plasma. Any degree of clotting is a positive test.
- Results can be reported across a range of 0 to 4+, 0 being negative (plasma remains liquid, no clot). A positive result of 4+ means that the plasma has hardened and the clot is not displaced when the tube is gently inverted (the consistency of an agar), due to strong coagulase activity.

Slide agglutination test:

- Clumps that will not mix uniformly into Plasma Coagulase represent a positive slide coagulase test and are indicative of *S. aureus*.
- A negative reaction is recorded when colonies mix smoothly into solution.
- Clumping in both the Plasma Coagulase and the control slides indicates that the organism autoagglutinates and is unsuitable for the slide coagulase test (tube coagulase test should be used as an alternative).

Storage and shelf life

- Store unopened at 2-8°C until expiry date shown on pack label.
- Reconstituted Plasma Coagulase can be stored at 2-8°C for 5 days.
- Alternatively it can be aliquoted into 0.5ml amounts, frozen promptly and stored at -20°C for up to 30 days.
- **DO NOT THAW AND RE-FREEZE.**

Quality control

The list below illustrates a range of performance control strains in routine use, which can easily be obtained as MAST QC STICKS™.

Test Organisms	Result
<i>Staphylococcus aureus</i> ATCC 9144 QC0831	Weak positive
<i>Staphylococcus aureus</i> ATCC 25923 QC0360	Positive
<i>Staphylococcus aureus</i> ATCC 33591 QC0496	Positive
<i>Staphylococcus epidermidis</i> ATCC 12228 QC0371	Negative

User quality control: Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect.

References

Bibliography available on request.