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MAST® ASSURE STAPHYLOCOCCAL COAGULASE TYPING ANTISERA

Intended Use
Liquid stable antisera for the determination of Staphylococcus coagulase types.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents
See pack label.

Formulation
MAST® ASSURE ANTISERA are prepared from rabbits hyperimmunised with standard strains of killed organisms possessing known serotypes or group specific antigens and contain 0.085% sodium azide as preservative.

Stability and storage
Store unopened at 2 to 8°C until the expiry date shown on the pack label. Once opened, MAST® ASSURE ANTISERA should be stored at 2 to 8°C and may be used until the expiry date given on the label. **Do not freeze reagents.**

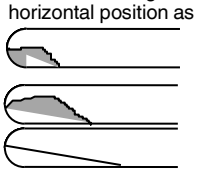
Warnings and precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, applicator sticks, clean glass test tubes, tube mixer, pipettes, swabs, MAST culture media, incinerators and incubators, etc., as well as specific items such as:
- sterile 0.85% saline solution
- autoclave capable of attaining 121°C or a device for heating bacterial suspensions to 100°C.
- centrifuge capable of achieving 3000 rpm.
- rabbit plasma
- normal rabbit serum
- diluent for rabbit plasma and serum. It is recommended that a 2% w/v peptone 1% w/v sodium citrate solution is used.

Procedure
A. Preparation of Antigen Solution.
1. Inoculate a colony of the test organism into 5ml of Mast Brain-Heart Infusion (BHI) Broth DM106 in a 30ml test tube and incubate at 37°C overnight to produce the coagulase enzyme.
2. After incubation centrifuge the culture fluid at 3000 rpm for 30 minutes, decant off the supernatant and use it as the antigen solution for the test.

B. Serotyping Procedure.
1. Add 0.1ml of antigen solution to each of 9 small test tubes.
2. To tube 1 add 0.1ml of type I antiserum, to tube 2 add 0.1ml of type II antiserum and to tubes 3-8 add 0.1 ml of type III-VIII antisera appropriately. To tube 9 add 0.1ml of 20-fold diluted normal rabbit serum.
3. Mix the contents of the tubes using a tube mixer then leave them standing in a 37°C incubator for 1 hour.
4. Remove the tubes from the incubator, add 0.2ml of 10-fold diluted rabbit plasma, mix the contents of the tubes using a tube mixer then place in the incubator at 37°C again.
5. Observe the tubes for coagulation after 1 hour incubation. If the results are unclear, continue the incubation and observe the results after 2 hours, 4 hours, 24 hours and 48 hours incubation.

Interpretation of results
1. To observe coagulation results hold the test tubes and tilt towards a horizontal position as shown below.



++ plasma coagulation

\+ production of insoluble material

\- no coagulation

2. Check that there is coagulation in the control tube. If there is no coagulation in the control tube extend the incubation time as detailed in section **B** step 5.
3. When coagulation is found in all tubes except one, the serotype of the coagulase of the test organism is taken as being the serotype corresponding to the antiserum in the tube where inhibition of clot formation has occurred.
   If inhibition is found in two or more tubes the incubation time should be increased and the results observed again when only one tube shows inhibition.
4. If after 48 hours coagulation is seen in the control tube and inhibition is seen in more than one tube the organism should be considered to have more than one coagulase specificity.

Limitations of use
Only cultures of organisms identified as *Staphylococcus aureus* by morphological and biochemical features should be serotyped with this product.
Note: Some strains of organisms may have a poor ability to produce coagulase resulting in difficulty in interpreting the results. If the tube to which normal rabbit serum has been added as a control shows no signs of coagulation after 24 hours, it is recommended that the coagulase production is enhanced by one of the following methods before retesting the strain.

Enhancement of Coagulase Production.
a) Shake culture.
1. Prepare shake flasks containing BHI Broth with up to 1/10th -1/5th the volume of the flask.
2. Inoculate a colony of the test organism into the culture medium and culture the organism aerobically at 37°C for 10 to 12 hours, shaking at about 120 rpm.
3. After incubation centrifuge the culture fluid at 3000 rpm for 30 minutes, decant off the supernatant and use it as the antigen solution for the test.

b) Rabbit plasma supplemented broth.
1. Prepare and sterilise some BHI Broth and add to it filter sterilised (0.22µm filter) rabbit plasma stock solution to give a final concentration of 10% v/v. Dispense 3ml of culture medium containing rabbit plasma to a small sterile test tube (about 10ml).
2. Inoculate a colony of the test organism into the culture medium and culture the organism aerobically at 37°C overnight.
3. Break up the coagulated culture medium and homogenise by pipetting up and down several times. Take the liquid part of the culture and use it as the antigen solution.

c) Agar plate containing rabbit plasma for selecting coagulase producing colonies.
1. Prepare and sterilise some Nutrient Agar and bring it to 50°C in a waterbath. Add to it filter sterilised (0.22µm filter) rabbit plasma stock solution to give a final concentration of 10% v/v and mix thoroughly. Pour into sterile plates and allow to set.
2. Inoculate plates with the test organism and culture the organism aerobically at 37°C overnight.
3. After incubation coagulase production may be observed if the plates are illuminated from below as a white ring around the colonies. The amount of coagulase produced is indicated by the diameter of the ring. Select a colony showing the largest ring an inoculate this into liquid medium as described in **b.** above.

Note: Some strains of organisms may produce a large amount of coagulase resulting in difficulty in interpreting the results. If all the tubes show coagulation after 1 hour incubation, the coagulase should be diluted according to the following method, then retested.
1. Prepare serial 2-fold dilutions of the test antigen solution from 1:2 to 1:16 using the diluent.
2. Add 0.1ml of the antigen solution at each dilution to 4 small test tubes respectively. Also add 0.1ml of 1:20 diluted normal rabbit serum to each tube, mix and incubate at 37°C for 1 hour.
3. Add 0.2ml of 10-fold diluted rabbit plasma to each tube, mix the contents of the tubes using a tube mixer then place in the incubator at 37°C for 1 hour and observe for coagulation.
4. Use the antigen solution for the test (section **B**) at the highest dilution that produces coagulation within 1 hour of incubation.

Note: Some strains of *S. aureus* produce the enzyme fibrinolysin which lyses coagulated plasma. If a coagulase test is performed using such a strain, coagulated plasma once formed may lyse again so care should be taken during observation of results.

Quality control
It is recommended that quality control should 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. Check for signs of deterioration. Do not use reagents if they are contaminated or cloudy.

References
Bibliography available on request.

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