DebenDiagnosticsLtd

STAPHYLOCOCCAL COAGULASE ANTISERA

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
RUO

Staphylococcus aureus (*S. aureus*) is a typical causal microorganism for purulent diseases such as wound infection and is a leading cause of food poisoning. It is also widely known as a cause of Staphylococcal Scalded Skin Syndrome (SSSS) and Toxic Shock Syndrome (TSS). Methicillin resistant *Staphylococcus aureus* (MRSA) is a known cause of nosocomial infections including serious sepsis and enteritis in patients with impaired immunological function or postoperative patients.

Free coagulase is produced extracellularly by *S. aureus* which coagulates human plasma or that of animals such as rabbits but is neutralised by antisera. This product is for typing of *Staphylococcal* coagulase and is prepared by hyperimmunising healthy rabbits with purified coagulase.

PRODUCTS

Staphylococcal Coagulase typing antisera: 5mL of serum in a bottle with dropper, each containing 0.08% w/v sodium azide preservative.

Set: 8 vials
Type I, II, III, IV, V, VI, VII, VIII
Each serum is also available individually.

INTENDED USE

The *Staphylococcus aureus* coagulase antisera are intended for serotyping research into prepared *Staphylococcus aureus* bacterial cultures, using tube agglutination to qualitatively detect the presence of serotype specific coagulase. *Staphylococcus aureus* coagulase antisera are intended for use by trained laboratory personnel.

This product is for research use only and must not be used in diagnostic procedures.

PRINCIPLE OF MEASUREMENT

Animal plasma coagulates when it is treated with coagulase produced by *S. aureus*, but this effect is inhibited if coagulase has been treated with specific antiserum. Typing is carried out based upon this principle.

PRECAUTIONS

1. **General precautions**
   1) Only bacteriological trained laboratory staff should handle the reagents.
   2) Reagents should only be used for the intended use.
   3) Reagents should be used according to the described procedures.
2. **Handling precautions**
   1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
   2) If reagent comes into contact with skin, mucous membranes or eyes, wash immediately with copious amounts of water and seek medical attention if necessary.
   3) Do not freeze the reagents or use past the expiration date as this may result in poor reagent performance.
   4) Reagents should be allowed to stand at 15 – 25ºC for at least 30 minutes before use.
   5) Used containers should not be used for other purposes.
   6) Sera with different lot numbers should not be mixed.
   7) Special precautions should be taken to ensure that the reagent caps are not exchanged.
   8) Avoid microbial contamination of opened reagent bottles. Do not use reagents if they are contaminated or cloudy.
3. **Precautions for disposal**
   1) The reagent contains 0.08% w/v sodium azide. Sodium azide may react with lead or copper pipes to form explosive heavy metal azides so the reagent should be disposed of with a large amount of water.
   2) All specimens, spills, inoculated products and equipment used in this test should be disposed of after treating by one of the following methods:
      [1] Soaking in 0.1% w/v hypochlorite for 1 hour or more.
      [2] Autoclave at 121°C for 20 minutes or more.

PROCEDURES

1. **Material required but not provided**
   Small test tubes, pipettes, micropipettes and tips, glass slides, glass pencil, test tube mixer, centrifuge, medium for coagulase production (Brain Heart Infusion liquid medium) auxiliary reagents for *Staphylococcal* Coagulase typing.
2. **Preparation of reagents**
   Ready to use.
3. **Specimens**
   Pure culture of *Staphylococcus aureus* identified by biochemical properties should be tested. If the specimen consists of multiple strains or is contaminated, it may not show correct results.
4. **Method**
   A) Preparation of test antigen
      1) A colony of the specimen is inoculated onto coagulase production media, and aerobically incubated at 37ºC overnight.
      2) Centrifuge the culture fluid at 3,000rpm for 30 minutes, and remove the supernatant to be used as the test antigen.
   B) Preparation of auxiliary reagents for *Staphylococcal* coagulase typing

Reagent	Preparation method	Prepared reagent	Storage after preparation
Normal rabbit plasma	Completely dissolved in 3mL purified water	Stock solution of normal rabbit plasma	Freeze remaining unused stock solution*
	Dilute the stock solution for the day 5-fold with diluent	Diluted normal rabbit plasma	Use on day of preparation
Diluent	Use as provided		
Normal rabbit serum	Dilute a required amount of serum 20-fold with diluent	Diluted normal rabbit serum	Store at 2-10ºC and avoid freezing

   * Once thawed do not refreeze.

   C) Coagulase typing test
      1) Prepare 9 small test tubes per specimen.
      2) Place 0.1mL test antigen solution in all 9 test tubes.
      3) Add 0.1mL type I antiserum to the first test tube, type II immune serum to the second test tube, repeating the process for the remaining types III to VIII. To the ninth test tube, add diluted normal rabbit serum as a control.
      4) Place the test tubes in a test tube-mixer and mix, then place in a water bath for one hour at 37ºC.
      5) After one hour, remove the test tubes from the water bath and add 0.2mL diluted normal rabbit plasma to each test tube. Mix the contents of each test tube with a test-tube mixer and allow to stand in a water bath for one hour at 37ºC.
      6) After one hour determine the results. If a reaction cannot be observed allow to stand for a further 2, 4, 24 or 48 hours before determination.
5. **Precaution for the test procedure**
   1. Determination of some strains may be difficult due to poor coagulase production of those organism strains. If coagulation in the control tube containing normal rabbit serum is not observed after 24 hours, the test should be repeated using test antigen cultured in the following media to increase coagulase production.
      A) Shaking culture:
         1) Prepare a shaking flask containing 1/5-1/10 flask volume of BHI liquid medium.
         2) Inoculate a colony of the specimen on BHI liquid medium, and aerobically culture at 37°C for 10·12 hours while shaking at about 120rpm.
         3) Centrifuge the medium by the above method and remove the supernatant as the test antigen.
      B) Inoculation in liquid medium containing rabbit plasma:
         1) Add 10% vol. of stock solution of normal rabbit plasma, which has been filter sterilised (0.22µm filter) to sterilised BHI liquid medium.
         2) Place 3mL rabbit plasma medium in small, sterilised test tubes (approx.10mL).

3) Inoculate a colony of the specimen and aerobically culture at 37°C overnight.
4) As the organisms proliferate, the medium coagulates.
5) Insert a pipette through the coagulated medium and collect the liquid. This liquid should be used as the test antigen.

C) Colony selection using rabbit plasma agar medium:
1) Heat-sterilise nutrient agar medium to 50°C and maintain temperature.
2) Add 10% vol. of stock solution of normal rabbit plasma, which has been filter sterilised (0.22µm filter) to the medium and mix.
3) Pour the solution into petri dishes to prepare culture plates. Inoculate the specimen onto the plate and aerobically culture at 37°C overnight.
5) After culturing, observe for the presence of a white ring around the colony while shining a light from the back of the petri dish.
6) Coagulase production of each colony is indicated by the size of ring. Inoculate a colony with the largest ring on the liquid medium by the method described in 'Inoculation in rabbit-plasma added liquid medium' to prepare the test antigen solution.

2. Determination of some strains may be difficult due to strong coagulase production.
If coagulation is observed in all test tubes after one hour, the test should be repeated after coagulase potency is adjusted by the following method.
1) Dilute the test antigen with diluent in the ratio of 1:2 to 1:16 using two-fold serial dilution.
2) Place 0.1mL of each diluted test antigen and 0.1mL diluted normal rabbit serum into test tubes, stir and allow to react at 37°C for one hour.
3) Add 0.2mL diluted normal rabbit plasma to the test tubes, stir and allow to react at 37°C for one hour. Observe for coagulation.
4) Dilute the test antigen to the maximum dilution possible in which coagulation is observed. Repeat the coagulase typing procedure.
3. Some strains of *S. aureus* produce the enzyme fibrinolysin which dissolves the fibrin in coagulated plasma. When such strains are used for the test, plasma will dissolve once coagulated.

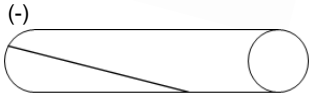
INTERPRETATION OF THE RESULTS

The results are determined according to the figure below.

(+) Plasma coagulation

(+) Fibrin deposit

(-)



1. Tilt the test tubes to observe coagulation of the contents.
2. Confirm coagulation in the control test tube. Prolong the reaction if no coagulation is observed.
3. When coagulation is observed in all but one of the test tubes, the serotype is determined as the one in which no coagulation is observed (coagulation inhibition).
If coagulation is inhibited in 2 or more test tubes, the reaction should be prolonged. The serotype is determined as the one in which coagulation inhibition is finally observed.
4. If coagulation is observed in the control tube after 48 hours, and inhibition is observed in multiple tubes, the coagulase type is interpreted as complex.

PERFORMANCE CHARACTERISTICS

1. Sensitivity
When coagulation is tested using coagulase produced by a reference strain of *Staphylococcus aureus*, 1MCD[1] coagulation of rabbit serum is inhibited.

2. Specificity
When the test is conducted with similar procedures to those of the sensitivity test, the corresponding coagulase produced by a reference strain of *Staphylococcus aureus* is only neutralised but other coagulases produced by other reference strains are not.

Dilute the supernatant in culture medium for *Staphylococcus* by two-fold serial dilution. Place 0.1mL diluted solution in small test tubes, add 0.1mL normal rabbit serum diluted 20 times and allow the mixture to react for one hour at 37°C.
Add 0.2mL rabbit plasma diluted 10 times to all small test tubes and allow the mixture to react for one hour at 37°c.
After the reaction, observe for coagulation. The highest dilution multiple of supernatant in which coagulation is observed is interpreted as the coagulase potency expressed as MCD/hr.

[1] MCD: Minimal Clotting Dose

STORAGE AND SHELF LIFE

Storage: 2-10°C
Shelf life: Up to the expiry date on the label.

REFERENCES

1) Supervised by the Ministry of Health, Labour and Welfare: Oral infection, Staphylococcal, Microbiological test manual, Bacterial and fungi tests, Third edition, Japan Public Health Association, D-133 (1987).
2) Supervised by the Ministry of Health, Labour and Welfare: Wound infection, Staphylococcal, Microbiological test manual, Bacterial and fungi tests, Third edition, Japan Public Health Association, J-15 (1987).
3) Supervised by the Ministry of Health, Labour and Welfare: Food Poisoning-Staphylococcal, Coagulase tests, Microbiological test manual, Bacterial and fungi tests, Second edition, Japan Public Health Association, 269 (1978).
4) Zenyoji, H. et al.: Staphylococcal coagulase typing (technique), Modern Media, Vol. 12 (12), 500 (1966).
5) Zenyoji, H. et al.: Staphylococcal coagulase-typing (applications), Modern Media, Vol. 13 (2), 39 (1966).
6) Shiota, H. et al.: Convenient coagulase typing for Staphylococcus aureus and its applications, annual report of Tokyo Metropolitan Institute of Public Health, 26, 1 (1975).

	Manufacturer
	Consult instruction for use
REF	Catalogue number
LOT	Batch code
RUO	For research use only – not for use in diagnostic procedures
	Storage temperature limitation
	Use by
LATEX	Contains or presence of natural rubber latex

DebenDiagnosticsLtd
Unit 4, West Road, Ransomes Europark, Ipswich Suffolk, IP3 9FD, United Kingdom
Tel. +44 (0) 1473 720869
Email: Info@debendiagnostics.co.uk
Web: www.debendiagnostics.co.uk

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