

Staphylococcal Coagulase Typing Antisera

INTENDED USE

Serotyping of *Staphylococcus aureus* coagulase.

SUMMARY AND EXPLANATION

Staphylococcus aureus is a typical causal microorganism for purulent diseases such as wound infection and also a leading cause of food poisoning. *S. aureus* is 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, extracellularly produced by *S. aureus* coagulates human plasma or that of animals such as rabbits. This effect is neutralized by antisera. Eight different sero-types are known for the organisms and neutralization test is used for typing of *S. aureus*.

The product is for typing of Staphylococcal coagulase, and prepared by hyper-immunizing healthy rabbits with purified coagulase.

PRODUCT

Staphylococcal Coagulase Typing Antisera are produced from rabbits and contain 0.08 w/v% sodium azide as a preservative. The following sera are provided in 5 mL vials.

Set: 8 vials/set

Type I, II, III, IV, V, VI, VII, VIII

PRINCIPLE

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) For in vitro diagnostic use only.
- 2) Only bacteriological trained laboratory staff should handle the reagents.
- 3) Reagents should only be used for the intended use.
- 4) 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 substance.
- 2) If reagent comes into contact with skin, eyes, or mouth wash immediately with copious amounts of water, 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°C-25°C 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 to form explosive heavy metal azides. The reagent should be disposed with copious amounts of water.
- 2) All specimens, spills, inoculated products and equipment used in this test should be treated 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.

TEST PROCEDURE

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 (Denka product marketed separately).

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 37°C overnight.
- 2) Centrifuge the culture fluid at 3,000 rpm 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 3 mL purified water	Stock solution of Normal rabbit plasma	Freeze remaining unused stock solution*
	Dilute the stock solution for the day with the diluent 5 fold	Diluted normal rabbit plasma	Use on the day of preparation
Diluent	Use as provided		
Normal rabbit serum	Dilute a required amount of serum with diluent 20 fold	Diluted Normal rabbit serum	Store at 2°C-10°C and avoid freezing

*Once thawed do not re-freeze.

C) Coagulase typing test

- 1) Prepare 9 small test tubes per specimen.
- 2) Place 0.1 mL test antigen solution in all 9 test tubes.
- 3) Add 0.1 mL 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 37°C.
- 5) After one hour, remove the test tubes from the water bath and add 0.2 mL 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 37°C.

- 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.

Precaution on test procedure

Determination of some strains may be difficult due to poor coagulase production of some strains of the organism. 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 120 rpm.
 - 3) Centrifuge the medium by the method described above, and remove the supernatant as the test antigen.
- B) Inoculation in liquid medium containing rabbit-plasma⁶⁾:
 - 1) Add 10 vol% of stock solution of Normal rabbit plasma, which has been filter sterilized (0.22 µm filter) to sterilized BHI liquid medium.
 - 2) Place 3 mL rabbit-plasma medium in small-sterilized test tubes (about 10 mL).
 - 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-sterilized nutrient agar medium to 50°C and maintain temperature.
 - 2) Add 10 vol% of stock solution of Normal rabbit plasma, which has been filter sterilized (0.22 µm filter) to the medium and mix.
 - 3) Pour the solution into petri dishes to prepare culture plates.
 - 4) 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.

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 0.1 mL of each diluted test antigen and 0.1 mL diluted Normal rabbit serum into test tubes, stir and allow to react at 37°C for one hour.
- 3) Add 0.2 mL 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 fibrinolysin an enzyme, which dissolves the fibrin in coagulated plasma. When such strains are used for the test, once coagulated plasma will dissolve.

INTERPRETATION OF RESULTS

The results are determined according to the figure below.

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.

(+) Plasma coagulation



(+) Fibrin deposit



(-)



PERFORMANCE CHARACTERISTICS

1. Sensitivity

When coagulation is tested using coagulase produced by a reference strain of *Staphylococcus aureus*, 1 MCD¹, 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 neutralized but other coagulases produced by other reference strains are not.

for one hour at 37°C.

Add 0.2 mL 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 MCD²/hr.

¹ MCD: Minimal Clotting Dose

STORAGE / SHELF LIFE

Storage: 2°C-10°C.

Shelf life: Up to the expiry date on the label.

PACKAGE

Staphylococcal Coagulase Typing Antisera: 5 mL serum vial with a pipette.

Set: 8 vials/set

Each serum is individually available.

Denka Seiken also supplies the following products individually.

Auxiliary Reagents for Staphylococcal Coagulase Typing

• Diluent	20 mL	1 vial
• Normal rabbit serum	3 mL	1 vial
• Normal rabbit plasma (lyophilized)	3 mL	1 vial

REFERENCE

- 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).

Please feel free to contact us at the following with your questions or comments :

TEL : +81-3-3669-9421 FAX : +81-3-3669-9390

¹ 1MCD: Minimal Clotting Dose

Dilute the supernatant in culture medium for *Staphylococcus* by two-fold serial dilution. Place 0.1 mL diluted solution in small test tubes, add 0.1 mL Normal rabbit serum diluted 20 times and allow the mixture to react

mbols



Batch code



Use by



In Vitro Diagnostic Medical Device



Temperature limitation (Store at)



Catalogue number



Consult Instruction for use



Contents of kit

YOKUJYO

MOB-4
EPAIT PIJG
CFIB JYbE

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 **DENKA SEIKEN CO., LTD.**

3-4-2 Nihonbashikayaba-cho, Chuo-ku, Tokyo, Japan

TEL : +81-3-3669-9421 FAX : +81-3-3669-9390

DENKA SEIKEN UK Ltd.

2 Coronation Lane
Oakthorpe, Swadlincote,
Derbyshire DE12 7QY
United Kingdom

TEL : +44 1530 270010 FAX : +44 1530 272009

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