IVD

Group-B Streptococci Typing Antisera

INTENDED USE

Serotyping of group B hemolytic streptococci.

SUMMARY AND EXPLANATION

Hemolytic streptococci are classified into 20 groups, A-V (I and J are omitted), according to serological difference in reaction to C-polysaccharide, a specific antibody of the organism. Group B streptococci (GBS): *Streptococcus agalactiae*, in particular, are known to cause serious infectious diseases including neonatal sepsis, pneumonia and meningitis resulting in high fatality rates and sequelae by secondary infection from the mother.

GBS serotype is identified by slide agglutination using heat-resistant polysaccharide antigens on the surface of the organism.

Group-B Streptococci Typing Antisera contains each specific agglutinin for the serotyping of GBS. The sera are prepared by hyper-immunizing healthy rabbits with each formalin-inactivated organism, heated at 56°C for 30 minutes, and removing cross agglutinins by absorption and aseptic filtration.

PRINCIPLE

When the reagent is mixed with group B hemolytic streptococci cells, which have antigens corresponding to the reagent, an antigen-antibody reaction occurs to produce agglutination. The reaction is macroscopically observed to determine each serotype.

PRODUCT

Group-B Streptococci Typing Antisera are produced from rabbits and contain 0.08 w/v% sodium azide as a preservative. The following sera are provided in 2 mL vials with pipette ready to use.

- Set: 6 vials/set
  Ia, Ib, II, III, IV, V
- TypeⅥ(NT6)
- TypeⅦ(7271)
- TypeⅧ(JM9)

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. Precaution on handling

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

Swine pancreas extract*1, phenol red solution*1, PH correction solution*1, Todd-Hewitt liquid medium, phosphate-buffered saline (PBS) pH7.2, small test tubes, pipettes (5 mL), micropipettes and tips, incubator (30°C), water bath (37°C), autoclave (121°C), centrifuge (900 × g or more), test tube mixer, bacteriological loops, glass slides, and glass pencils.

*1 : products of Denka Seiken separately marketed.

2. Preparation of reagents

Ready to use.

3. Specimens

Pure culture of group B hemolytic streptococci 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) Inoculate a specimen on Todd-Hewitt liquid medium and culture at 30°C for 16-20 hours.
2) Prepare the test antigen according the procedure described below.
   a. Place the culture medium in a small test tube and centrifuge at 900 × g for 20 minutes.
   b. Remove the supernatant and add 0.5 mL Todd-Hewitt liquid medium, 200 μL Swine pancreas extract, and a drop of Phenol red solution to the precipitate, and shake vigorously.
   c. Add the PH correction solution to adjust the liquid to pH8.0-8.5 (reddish-purple color).
   Place in a water bath at 37°C for an hour while shaking occasionally. If decrease in pH (orange to yellow color of the liquid) is noted, PH correction solution should be added as necessary.
   d. After completion of the reaction, centrifuge the mixture at 900 × g for 20 minutes. Remove the supernatant and add 0.5 mL PBS. Uniformly suspend the precipitate using a pipette or test tube mixer and heat the suspension at 121°C for 30 minutes.
   e. Cool and centrifuge at 900 × g for 20 minutes. Remove the supernatant, add 0.5 mL PBS and uniformly suspend using a pipette or test tube mixer. This suspension is used as the test antigen.
   f. In order to exclude possible spontaneous agglutination, the test antigen should be mixed with physiological saline on a slide to confirm that there is no spontaneous agglutination. If spontaneous agglutination is seen, another colony should be selected.

B) Slide agglutination

1) Place a drop on antiserum and physiological saline(30 μ L) another section of the partitioned slide.
2) Place a test antigen(5-10 μ L) on the area above each drop of serum or physiological saline, and mix well using the loop.
3) Tilt the glass slide back and forth and observe for agglutination.
4) Check whether spontaneous agglutination occurs with the physiological saline.

5) Only strong agglutination observed within 1 minute in the reaction with antiserum should be regarded as positive. Delayed or weak agglutination is regarded as negative.

Precaution on test procedure

1. Care should be taken when adjusting the pH of the solution.
2. Test antigen is preserved in sodium azide to a concentration of 0.1 w/v% at 2°C-10°C, and are viable for a month.
3. Prepare a defatted and flawless glass slide.
4. Use a separate loop, flame sterilized loop, or pipette tip for each serum to avoid cross-contamination among sera during the slide agglutination procedure.

INTERPRETATION OF RESULTS

Agglutination can be grossly observed using an indirect light source over a darkened background. It should be confirmed that no agglutination is found in the control (reaction with test antigen and physiological saline). Only strong agglutination observed within 1 minute of the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative (refer to the table below interpretation).

Results of monovalent serum	Determination
Only one antiserum tests positive.	Name of antiserum that tested positive is interpreted as the serotype of the specimen.
Multiple antisera test positive.	The specimen is determined to possess multiple antigen types.
All antisera tests negative.	Serogrouping and serotyping are determined to be impossible.

Precautions in Interpretation

1) When agglutination is observed between the test antigen and physiological saline, the test should be repeated selecting another colony.
2) When multiple antisera give positive results, the specimen may not be derived from an axenic culture or may have multiple serotypes.
3) When agglutination is not observed with any of the antisera, the specimen may not contain the antigens of Group-B Streptococci Typing antisera or may shared antigens.

PERFORMANCE CHARACTERISTICS

1.Sensitivity test

When a drop of the product is reacted on a glass slide with a reference strain of a known serotype, granular agglutination is observed.

2.Specificity test

When the product is tested according to the similar manner to the sensitivity test, agglutination is observed in the reaction with a reference strain of corresponding serotype, but not observed in the reaction with a reference strain of a different serotype.

STORAGE / SHELF LIFE

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

PACKAGE

Group-B Streptococci Typing Antisera: 2 mL serum vials with pipette.

- Set: 6 vials/set
  Each serum is individually available.
- Type Ⅵ (NT6)
- Type Ⅶ (7271)
- Type Ⅷ (JM9)

Denka Seiken also supplies the following products individually.

- Auxiliary Reagents for Hemolytic Streptococcus Typing

Contents:			
Swine pancreas extract	5 mL	4 vials
Phenol red solution	5 mL	1 vial
PH correction solution	5 mL	2 vials

REFERENCE

1) Lancefield, R.C., *et al.*: A serological differentiation of human and other groups of hemolytic streptococci, J. exp. Med. 57, 571 (1933)
2) Senmura, T.: Pregnancy and GBS, Obstetrics and Gynecology, 57, 179 (1976)
3) Jelinkova.: Type identification of group B streptococci, Prague (1976)
4) Takizawa, K., *et al.*: Serological classification of Streptococcus agalactiae, Medical Technology, 10, 236 (1982)
5) Yokoo, H., *et al.*: Serotype of group B hemolytic streptococci, Clinical Bacteriology, 11, 239 (1984)
6) Supervised by the Ministry of Health, Labour and Welfare: Streptococci, Microbiological test manual, Bacterial and fungi tests, Third edition, Japan Public Health Association, F-2 (1987)

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

Batch code


Use by

In Vitro Diagnostic Medical Device


Temperature limitation (Store at)

Catalogue number


Consult Instruction for use

Contents of kit

CE

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