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1990.2 Updated

溶血レンサ球菌群別及び型別用免疫血清

In Vitro Diagnostic Reagents
Ref. (63E) No. 1765, 1766, 1767

Hemolytic streptococcus Grouping and Typing Immune Sera

The grouping of Hemolytic streptococcus into 20 groups from A to V excluding I and J, has been made according to the serological differences of C-polysaccharides possessed by the organism. Among the groups, A, B, C and G have human-pathogenicity and are considered to be epidemiologically important.

These sera are immune sera used for serotyping of Hemolytic streptococcus and are liquid products which contain group-specific or type-specific antibodies.

The antisera are prepared by hyper-immunizing rabbits with bacterial strains having group-specific or type-specific antigens. After bleeding, the serum is separated, heated at 56℃ for 30 minutes, absorbed to remove cross-agglutinins and sterilized by antibacterial filtration. As a preservative, sodium azide is added at 0.1 w/v%.

FEATURES

The grouping and typing of the bacteria which are performed on a glass slide using the products has the following features based on an advance pancreatic extract treatment for bacterial cells. Removal of spontaneous agglutination which is specific for coccus. Easy operation compared with other methods such as gel diffusion. Result within a short time.

PRODUCTS

Hemolytic streptococcus Grouping Immune Sera "SEIKEN" Ref. (63E) No. 1767
Each serum contains specific agglutinins to C-polysaccharides of homologous group.
Set 4 vials: Group A, B, C and G

Hemolytic streptococcus Typing Immune Sera for Group A (T-typing) "SEIKEN"
Ref. (63E) No. 1765
Each serum contains type-specific agglutinins against T-protein of group A.
Set 24 vials: Polyvalent 5 vials (T, U, W, X, Y)
Monovalent 19 vials (See the table below)

T-Polyvalent sera	T-agglutinins contained in the serum			
T	1	3	13	B3264
U	2	4	6	28
W	5/27/44		11	12
X	8	14/49	25	Imp. 19
Y	9	18	22	23

Homolytic streptococcus Typing Immune Sera for Group B "SEIKEN"
Ref. (63E) No. 1766
Each serum contains type-specific agglutinins against group B streptococci.
Set 6 vials: Ⅰa, Ⅰb, Ⅱ, Ⅲ, Ⅳ, Ⅴ

INTENDED USE

Determination of Hemolytic streptococcus group and sero-type.

PRINCIPLE

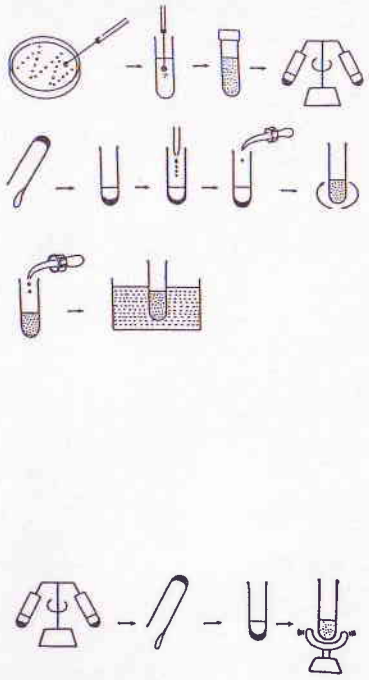
When the serum which contains a specific antibody against each antigen type and group is mixed with Hemolytic streptococcus cells, an aggregate of the bacteria is formed by the antigen-antibody reaction which produces a stitch-like formation of bacteria cells and antibody molecules. The bacterial antigen is identified by this aggregate as giving a positive result.

PREPARATION OF SPECIMENS, REAGENTS AND EQUIPMENT

1. **Specimen:**
   Test bacteria isolated on blood-agar plate from patient's material
2. **Reagents:**
   Todd-Hewitt broth
   Swine pancreatic extract*
   Phenol red solution*
   Solution for pH adjustment*
   Phosphate-buffered saline (PBS) pH 7.2
   *These are available from our firm.
3. **Equipment:**
   Glass slide, platinum wire, platinum loop (internal diameter 3 mm), small test tube, pipette or micromixer, water bath, centrifuge

PROCEDURE

1. **Agglutination test**
   [Preparing test bacteria]



1) A colony of the isolated streptococci is inoculated in 5 ml of Todd-Hewitt broth, and the broth is incubated at 29－30 ℃ overnight, and centrifuged at 3000 rpm for 20 minutes.
2) The supernatant is discarded and 0.5 ml of Todd-Hewitt broth, 4 drops of swine pancreatic extract and 1 drop of Phenol-red solution as pH indicator are added to the sediment, and the mixture is shaken well.
3) Then pH of the mixture is adjusted to 8.0－8.5 by adding the solution for pH adjustment until the color becomes reddish purple, and the mixture is incubated at 37 ℃ for 1 hour for the digestion of the organisms. If a change of color to scarlet or yellow is recognized after 15 minutes digestion, indicating a remarkable decline in pH, the solution for pH adjustment should be added accordingly. To attain homogeneous digestion, occasional sufficient shaking is necessary.
4) The suspension is centrifuged, and the supernatant is discarded, then 0.5 ml of PBS is added to the sediment and a homogeneous suspension is made

using a pipette or a micromixer.

5) Then, to prevent spontaneous agglutination of the antigen suspension itself, agglutination should be done each time against physiological saline to ensure that it is nonagglutinable.
6) If the antigen suspension is inadequate for use due to strong spontaneous agglutination, 4 drops of swine pancreatic extract is added additionally and the digestion is carried out at 50 ℃ for another 20 minutes.
7) If the antigen suspension is not yet appropriate for use, another selection of colonies is necessary

[Grouping test]

1. One drop of grouping serum A, B, C or G and one loopful of the digested bacterial suspension are mixed thoroughly on a glass slide, and the results are observed with the naked eye.
2. A clear-cut agglutination is to be observed within one minute between the corresponding (homologous) antigen and antibody.

[Typing test]

1. Group A T-typing test

1) If the group A streptococcus was identified by the grouping test, then similar agglutination tests are further processed on a glass slide using the same antigen suspension against T-typing polyvalent sera.
2) If a positive reaction occurs with one of these polyvalent sera, an agglutination test is carried out using T-typing monovalent sera which are the constituents of the polyvalent serum to determine the type of T-antigen.

2. Group B typing test

If the group B streptococcus was identified by the grouping test, the typing test is carried out as follows:

1) Heat pancreas-extract treated bacterial suspension at 120 ℃ for 30 minutes, and agglutination tests are carried out using the heated suspension on a glass slide with 6 kinds antisera (i.e. Ⅰa, Ⅰb, Ⅱ, Ⅲ, Ⅳ, Ⅴ).
2) When a strong agglutination is seen within one minute, the serotype of the serum is that of the bacteria specimen. The serotype which is identified by this procedure corresponds to the serotype identified by the WHO standard precipitation test, which uses sulphic extracted antigen.

NOTE

1. Bacterial antigen which has little spontaneous agglutination can be obtained under the incubation temperature at 30 ℃ rather than 37 ℃ when the test bacteria is cultivated.
2. Adjust the pH of the digesting bacterial suspension carefully in order not to overrun.
3. Although T protein of group A is resistant for pancreas extract digestion, the extension of the digestion time will cause gradual sublation and reduction of agglutinativity.
4. Digested bacterial cells can be used again for up to one month when refrigerated after washing the cell suspension a few times with PBS and resuspending it with a small volume of PBS containing 0.01 w/v% sodium azide.
5. Use clean delipidized glass slides without scratches for the agglutination test.
6. Note that the T-typing agglutination of group A streptococcus occasionally requires longer reaction time than the grouping agglutination test.

PRECAUTIONS

1. In agglutination tests, the platinum wire should be sterilized by a flame before moving to the next section on the glass slide, to avoid mixing of different antisera.
2. Live cells and instruments such as slides and test tubes used for the tests should be disposed of after soaking in 0.1% sodium hypochlorite solution (available chlorine approximately 1000 ppm) for 1 hour or more, or after autoclaving at 121℃ for 20 minutes or more.
3. Freezing of sera may sometimes produce a precipitate after thawing.
4. As this reagent contains sodium azide it may react with lead or copper to form explosive heavy metal azides. So discard it using a large quantity of water.

STORAGE AND SHELF LIFE

Storage: 2−10 ℃

Shelf life: Up to expiry date on the label.

PACKAGE

Each 2 ml in a vial with a pipette

Hemolytic streptococcus Grouping Immune Sera "SEIKEN"
Set 4 vials

Hemolytic streptococcus Typing Immune Sera for Group A (T-typing) "SEIKEN"
Set 24 vials

Hemolytic streptococcus Typing Immune Sera for Group B "SEIKEN"
Set 6 vials

*Following reagents are available from us.

Hemolytic Streptococcus Antigen Processing Reagents

Product name	Contents
Swine pancreatic extract 5 ml	Extracts from swine fresh pancreas with ethanol.
Solution for pH adjustment 5 ml	Containing sodium carbohydroxide by 2.65 w/v% (0.25 M) and Tris (hydroxymethyl) amino-methane by 1.21 w/v% (0.1 M).
Phenol red solution 5 ml	Containing Phenol red by 0.02 w/v% and sodium azide by 0.1 w/v%.

Set:
Swine pancreatic extract 5 ml × 4
Solution for pH adjustment 5 ml × 2
Phenol red solution 5 ml × 1

REFERENCES

1. Takizawa, K., et al.: Reexamination and Characterization of the T-Agglutination Complex or Pattern of *Streptococcus pyogenes*: Preparation of anti-T Factor Sera. Japan. J. Microbiol., **14**, 269 (1970).
2. Cowan, S. T., et al.: Manual for the Identification of Medical Bacteria, Cambridge University Press (1965).
3. Jelinkova, J.: Type Identification of Group B Streptococci, Prague (1976).

DENKA SEIKEN CO., LTD.
3-4-2 Nihonbashikayaba-cho, Chuo-ku, Tokyo, Japan