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緑膿菌免疫血清「生研」
In Vitro Diagnostic Reagents
Ref. (62E) No.1477

Pseudomonas aeruginosa Antisera "SEIKEN"

Pseudomonas aeruginosa is a bacterium of gram negative bacillus with monotrichate flagellum and motility and is also important due to its effect as a cause of so-called opportunistic infection.

These products are O-sero grouping sera which are used for the serogrouping of *P. aeruginosa*. The products are liquid sera which contain specific antibodies against O-group antigens. When they are used, O-group will be identified by slide agglutination.

The antisera are prepared by immunizing rabbits with antigens prepared from reference strains. For immunization, the strains are heated at 100 ℃ for 60 minutes. Reference strains are determined according to the serological methods prescribed by the committee for taxonomy of *P. aeruginosa* in Japan. After bleeding, the serum is separated, inactivated at 56 ℃ for 30 minutes, absorbed to remove non-specific agglutinins and filtered under sterile conditions. As a preservative, sodium azide is added by 0.1 w/v%.

The committee for serological grouping held by the study group for *P. aeruginosa* in Japan employed the slide agglutination test as well as tube agglutination test. The merit of this method is in using live-cells as antigens. Seventeen groups of sera developed by Homma et al. were newly divided into 13 groups of sera.

Group antigens are designated as A - M (Please refer to the attached table) without using numbers, in order to avoid confusion with the sero-typing antigen numbers which have been used internationally. The committee decided to add group N in 1982.

PRODUCTS

Set 17 vials
Polyvalent sera 3 vials
Polyvalent Ⅰ (A, C, H, I, L-group)
Polyvalent Ⅱ (B, J, K, M-group)
Polyvalent Ⅲ (D, E, F, G, N-group)
Grouping sera 14 vials A - N-group

PURPOSE

Determination of *Pseudomonas aeruginosa* sero-group

PRINCIPLE of MEASUREMENT

This product is mixed with *Pseudomonas aeruginosa* to cause an antigen-antibody reaction and forms an aggregate of the bacteria which is observed macroscopically. The sero-group is determined using this principle.

PROCEDURE

After the identification as *P.aeruginosa*, the serological procedure should be applied using the slide agglutination test.

Firstly, confirm an agglutination according to the procedure shown below, using polyvalent sera. Secondly, when an agglutination occurs with a polyvalent serum, confirm agglutination by the same procedure using each monovalent grouping serum which is included in the polyvalent serum.

In the agglutination test, live-cells should be directly used as antigens, but in the case of a negative or slight reaction it is necessary to test again with antigens heated at 120 ℃ for 90 minutes. Grouping tests should be done immediately after the isolation, as colonies of *P. aeruginosa* easily tend to dissociate.

1. Prepare a clean glass slide, border it all around and partition into several parts with a glass-pencil and put a drop of serum onto the center of each part of the glass slide with the pipette provided with the serum vial and put a drop of saline onto the center of the control part of the glass slide.
2. Suspend live-cells of so-called 1a-type colony with saline and make a density cell suspension. Put one loopful of the antigenic dense suspension onto the vicinity of the drops of serum or saline and mix the antigen and serum or antigen and saline well using a bacteriological loop.
3. Tilt the glass slide back and forth and then observe the agglutination pattern. In addition to this, as a control, confirm whether spontaneous agglutination occurs or not with the reaction of the antigen and saline.

READING

The results are read as follows:

Saline-antigen solution reaction	Antiserum-antigen solution reaction	Judgment
Spontaneous agglutination (－)	Strong agglutination within 1 minute	Positive (＋)
	No agglutination within 1 minute	Negative (－)
Spontaneous agglutination (＋)	Reservation of judgment	

1. When the reaction is unclear or slight, or occurs later than one minute after treatment, or the antigen agglutinates with more than two specific sera, the test should be repeated using cells heated at 121 ℃ for 90 minutes as the antigen.
2. If positive agglutination is observed, the isolate contains the O-antigen of that specific serological group.
3. When the antigen agglutinates strongly with more than two specific group sera, the effect should be recorded. If neither live cells nor heated cells show agglutination, a new O-antigen is assumed to be present.
4. If agglutination is observed in the saline control, the test should be repeated selecting another colony.

EFFICIENCY

1. **Sensitivity**

   When one drop of this antiserum is allowed to react on a slide with a known serotype of the reference strain, macroscopically granular agglutination is observed.

2. **Specificity**

   In a test carried out in the same manner as the sensitivity test, this antiserum agglutinates with only the reference strain corresponding to the serotype, while in reactions with non-corresponding reference strains, macroscopic agglutination is not observed.

PRECAUTIONS IN USE AND TREATMENT

1. Live cells and instruments such as slides and test tubes used for the tests should be disposed of after soaking in 0.1w/v% sodium hypochlorite solution (available chlorine approximately 1000ppm)for 1 hour or more, or after autoclaving at 121 ℃ for 20 minutes or more.
2. Freezing of sera may sometimes produce a precipitate after thawing.
3. 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 the expiry date on the label.

PACKAGE

Each in 2mL vial with a pipette

1 set 17 vials

REFERENCE

1. Homma, J. Y.: Designation of the Thirteen O-group Antigens of *Pseudomonas aeruginosa*; An Amendment for the Tentative Proposal in 1976 Japan. J. Exp. Med., **52**, 317 (1982).

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