

PSEUDOMONAS AERUGINOSA ANTISERA

Pseudomonas aeruginosa (P. aeruginosa) is a gram-negative bacillus with monotrichate flagellum and motility. It is important due to its effects caused by so-called opportunistic infection.

These products are liquid sera which contain specific antibodies against O-group antigens and are used for O-serogrouping of *P. aeruginosa* by slide agglutination. The antisera are prepared by immunising rabbits with antigens prepared from reference strains that have been heated at 100°C for 60 minutes. After bleeding, the serum is separated, inactivated at 56 °C for 30 minutes, absorbed to remove non-specific agglutinins and filtered under sterile conditions.

PRODUCTS

Seventeen groups of sera developed by Homma et al. were divided into 13 groups of sera. Group antigens are designated as A - M without using numbers in order to avoid confusion with the internationally used serotyping antigen numbers. Group N was further added in 1982.

P. aeruginosa antisera: 2mL serum in a bottle with dropper.

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

Each serum is also available individually.

INTENDED USE

The *Pseudomonas aeruginosa* antisera are intended for serogrouping research into prepared *Pseudomonas aeruginosa* bacterial cultures, using slide agglutination to qualitatively detect the presence of bacterial antigens. *Pseudomonas aeruginosa* 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

This product is mixed with *P. aeruginosa* to cause an antigenantibody reaction and form an aggregate which is observed macroscopically to determine the serogroup.

PRECAUTIONS

1. Handling precautions

- All specimens, samples and containers coming into contact with samples should be treated as infectious.
- If reagent comes into contact with skin, mucous membranes or eyes, wash immediately with copious amounts of water and seek medical attention if necessary.
- Do not use reagents past the expiration date as this may result in poor reagent performance.
- Freezing of sera may sometimes produce a precipitate after thawing.

2. Precautions for disposal

- 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 disposed of after treating by one of the following methods:
 - [1] Soaking in 0.1% w/v hypochlorite for 1 hour or more. (Available chlorine approximately 1000ppm)
 - [2] Autoclave at 121°C for 20 minutes or more.

PROCEDURES

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. When an agglutination occurs with a polyvalent serum, confirm agglutination by the same procedure

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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°C for 90 minutes. Grouping tests should be done immediately after the isolation as colonies of *P. aeruginosa* tend to dissociate easily.

- Prepare a clean glass slide, border it all around and partition into several parts with a glass-pencil. Put a drop of serum onto the centre of each part of the glass slide with the pipette provided with the serum vial and put a drop of saline onto the centre of the control part of the glass slide.
- Suspend live cells of a '1a' type colony with saline and make a
 dense 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.
- Tilt the glass slide back and forth and then observe for 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.

INTERPRETATION OF THE RESULTS

The results are read as follows:

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

- When the reaction is unclear or slight, 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 °C for 90 minutes as the antigen.
- If positive agglutination is observed, the isolate contains the Oantigen of that specific serological group.
- 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.

PERFORMANCE CHARACTERISTICS

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.

STORAGE AND SHELF LIFE

Storage: 2-10°C

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

REFERENCES

 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).





