BIO-RAD

ANTISERUM PSEUDOMONAS AERUGINOSA

SEROTYPING OF PSEUDOMONAS AERUGINOSA

IVD

1- CLINICAL VALUE

In hospital, *Pseudomonas aeruginosa* infections can take on epidemic proportions. This very serious situation may require temporary closure of the department in which these infections occur. It is essential to investigate the epidemiological pathway of these hospital infections, to identify their origin (very often so-called "aseptic" solutions used for disinfection of non-autoclavable instruments and catheters), or to exclude the responsibility of the nursing staff, possibly healthy carriers (throat, stools) of *Pseudomonas aeruginosa* not responsible for the epidemic.

This epidemiological survey must determine whether or not all of the strains of *Pseudomonas aeruginosa* isolated during the epidemic are identical.

Following identification of the species, determination of the O-antigen group provides essential and sometimes sufficient information for epidemiological surveys.

2- INTENDED USE

P. aeruginosa antisera are used for serological identification of cultures of *P. aeruginosa* by the slide agglutination method, for epidemiological purposes.

3- PRINCIPLE

The test is based on agglutination, by specific sera, of bacteria possessing the corresponding antigens.

Anti-Pseudomonas aeruginosa (anti-O) agglutinating sera

According to the classification established by Habs (1) and completed by the International *Pseudomonas* Sub-Committee, there are 16 O-antigen groups, numbered from 1 to 16.

These antisera are obtained by immunizing rabbits with heated bacterial suspensions (which destroys heat-labile H-antigen). Specificity is then determined by cross-saturation.

1) Monovalent sera

16 monovalent sera numbered from 1 to 16 are available.

Strong cross-reactions are observed between groups O:2 and O:5, and between groups O:7 and O:8 and between groups O:13 and O:14. Monovalent sera corresponding to these groups are consequently absorbed.

2) Polyvalent sera

To facilitate typing, 4 serum mixtures are prepared and designated by the acronyms PMA, PME, PMC and PMF.

They have the following compositions:

PMA = P1 + P3 + P4 + P6 PME = P2 + P5 + P15 + P16

PMC = P9 + P10 + P13 + P14 PMF = P7 + P8 + P11 + P12

4- HOW SUPPLIED

Polyvalent sera and monovalent sera are supplied in 3 ml dropper bottles (60 tests).

• Polyvalent sera

Antiserum Pseudomonas aeruginosa polyvalent PMA	Code : 58922	Antiserum Pseudomonas aeruginosa polyvalent PMC	Code : 58942
Antiserum Pseudomonas aeruginosa polyvalent PME	Code : 58932	Antiserum Pseudomonas aeruginosa polyvalent PMF	Code : 58952

• Monovalent sera

Antiserum Pseudomonas aeruginosa monovalent P1	Code : 58901	Antiserum Pseudomonas aeruginosa monovalent P9	Code : 58909
Antiserum Pseudomonas aeruginosa monovalent P2	Code : 58902	Antiserum Pseudomonas aeruginosa monovalent P10	Code : 58910
Antiserum Pseudomonas aeruginosa monovalent P3	Code : 58903	Antiserum Pseudomonas aeruginosa monovalent P11	Code : 58911
Antiserum Pseudomonas aeruginosa monovalent P4	Code : 58904	Antiserum Pseudomonas aeruginosa monovalent P12	Code : 58912
Antiserum Pseudomonas aeruginosa monovalent P5	Code : 58905	Antiserum Pseudomonas aeruginosa monovalent P13	Code : 58913
Antiserum Pseudomonas aeruginosa monovalent P6	Code : 58906	Antiserum Pseudomonas aeruginosa monovalent P14	Code : 58914
Antiserum Pseudomonas aeruginosa monovalent P7	Code : 58907	Antiserum Pseudomonas aeruginosa monovalent P15	Code : 58915
Antiserum Pseudomonas aeruginosa monovalent P8	Code : 58908	Antiserum Pseudomonas aeruginosa monovalent P16	Code : 58916

5- STORAGE

Sera stored at +2-8°C in the absence of contamination are stable until the expiry date indicated on the kit (even when opened).

6- MATERIAL REQUIRED BUT NOT SUPPLIED

- Glass slide.
- Plastic or platinum inoculation loop.
- Physiological saline.

7- PRECAUTIONS FOR USE

- Always comply with current techniques and precautions concerning protection against microbiological hazards, for handling and elimination of material and biological products used for the agglutination reaction.
- These sera contain < 0.1% sodium azide. Sodium azide can react with lead or copper present in the plumbing forming explosive metallic azides. When eliminating these reagents, rinse abundantly with water to avoid the formation of azide deposits.
- Do not dilute reagents.

8- PROCEDURE

Serotyping is performed after identification of the species on a fresh, pure culture of *P. aeruginosa* isolated on non-selective agar medium.

Perform a control test on the strain to be tested in physiological saline:

- Take one loop of culture.
- Suspend these bacteria in a drop of physiological saline, ensuring homogeneous suspension.

No agglutination should be observed with physiological saline. If agglutination is observed, it correspond to a self-agglutinating strain and the test with antisera cannot be performed.

- Start by testing agglutination with polyvalent sera, then with the specific sera corresponding to the mixture giving marked agglutination.
- Deposit 1 drop of immune serum on the slide.
- Take one loop of fresh, pure culture of *P. aeruginosa*.
- Suspend these bacteria in a drop of serum, ensuring homogeneous suspension gradually adding bacteria to the serum.
- Shake the slide with a gently rotary movement.
- Examine the mixture with the naked eye over a dark surface or over a concave mirror.

9- INTERPRETATION OF THE RESULTS

A positive reaction corresponds to the appearance of agglutination in a **maximum of 5 minutes**. O-antigen agglutination is fine and regular and is very easily distinguished from any poorly emulsified fragments of bacterial colony.

Note : *: In the case of failure (for example with strains producing a lot of mucosal antigens), it is recommended to prepare a very thick suspension in distilled water from a fresh, pure culture on agar and heat this suspension at 120°C for 30 minutes (autoclave). This suspension is centrifuged, the supernatant is eliminated, and the test is performed on the bacterial pellet.*

10-TEST PERFORMANCES/QUALITY CONTROL

The activity of each *Pseudomonas aeruginosa* agglutinating serum is controlled with the following panel of reference strains:

Strains	Serotype
Pseudomonas aeruginosa CIP 59.33 / ATCC 33348	P1
Pseudomonas aeruginosa CIP 59.34 / ATCC 33349	P2
Pseudomonas aeruginosa CIP 59.35 / ATCC 33350	P3
Pseudomonas aeruginosa CIP 59.36 / ATCC 33351	P4
Pseudomonas aeruginosa CIP 59.37 / ATCC 33352	P5
Pseudomonas aeruginosa CIP 59.39 / ATCC 33354	P6
Pseudomonas aeruginosa CIP 59.38 / ATCC 33353	P7
Pseudomonas aeruginosa CIP 59.40 / ATCC 33355	P8
Pseudomonas aeruginosa CIP 59.41 / ATCC 33356	P9
Pseudomonas aeruginosa CIP 59.43 / ATCC 33357	P10
Pseudomonas aeruginosa CIP 59.44 / ATCC 33358	P11
Pseudomonas aeruginosa CIP 59.45 / ATCC 33359	P12
Pseudomonas aeruginosa CIP 60.92 / ATCC 33360	P13
Pseudomonas aeruginosa CIP 72.12	P14
Pseudomonas aeruginosa CIP 72.13	P15
Pseudomonas aeruginosa CIP 74.21	P16

11-QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

12-LIMITS OF USE

- Identification of the bacterial species must be performed before determination of the serogroup.
- About 5% of strains of *Pseudomonas aeruginosa* are unstable and self-agglutinating.
- About 1% of stable strains of *Pseudomonas aeruginosa* belong to groups other that 1 to 16. Typing these new O groups appears to be of limited practical value.

13-REFERENCES

1. HABS I. Z. Hyg., **144**, 218-228.
2. VIEU J.F., ALLOS G., HASSAN-MASSOUD B., SANTOS-FERREIRA M.O. et TSELENTIS G.. Existe-t-il une épidémiologie géographique des sérogroupe O de *Pseudomonas aeruginosa*. Bull. Soc. Path. Ex., 1984, **77**, 288-294.

Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette France
Tel. : +33 (0) 1 47 95 60 00
Fax : +33 (0) 1 47 41 91 33

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