

PSEUDOMONAS-CF-IgG

Antigen and standard antiserum



STATENS
SERUM
INSTITUT

5 Artillerivej
2300 Copenhagen S
Denmark

Information and ordering

SSI Diagnostica
2 Herredsvejen
3400 Hillerød
Denmark
Tel.: +45 4829 9178
Fax: +45 4829 9179
microbiology@ssi.dk
www.ssi.dk

Pseudomonas-CF-IgG antigen and standard antiserum are for the quantitative measurement of the antibody level of *P. aeruginosa* in human serum samples.

Application

Chronic *P. aeruginosa* infection can reliably be discriminated from intermittent colonization by measuring serum IgG antibodies against *P. aeruginosa*. During the chronic infection a pronounced and increasing antibody response develops whereas this is not the case in intermittently colonized patients. The level of the antibody response in chronically infected patients correlates to the severity of the infection.

Description

The Pseudomonas-CF-IgG antigen is supplied with a vial containing 9 mg lyophilized antigen obtained by sonication of the 17 most common *P. aeruginosa* serotypes (O-1 through O-17). The Pseudomonas-CF-IgG standard serum vial contains 100 µL pooled human standard serum.

Principle

The Pseudomonas-CF-IgG antigen is used as a coating agent in a traditional ELISA setup. More than 64 different antigens are detectable in the antigen pool. The results from the Pseudomonas-CF-IgG standard serum are used to calculate the concentration of antiserum in the patient sample.

Limitations

Chronic infections with related Gram-negative bacteria e.g. *Burkholderia* may give rise to cross-reactive antibodies (false positive results).

Materials required but not provided

Sterile distilled water
Maxisorp ELISA plates (NUNC®)
Normal human serum
Coating buffer (1.724 g NaH₂PO₄, H₂O + 13.40 g Na₂HPO₄, 12 H₂O + 42.35 g NaCl + 5000 mL H₂O)
Washing buffer (2000 mL coating buffer + 2 mL Tween 20)
Dilution buffer (1000 mL coating buffer + 1 mL Tween 20 + 15g NaCl)
Rabbit-Anti-Human IgG HRP (DAKO® P0214)
2N H₂SO₄
TMB Plus Standard (Kem-En-Tec Diagnostics)

Procedure

Controls and unknown should be assayed in duplicates. Normal human serum is used as negative control.

1. The Pseudomonas-CF-IgG antigen vial is reconstituted with 100 µL sterile distilled water. Dilute the amount to be used the same day 1:2000 with coating buffer.
2. Add 100 µL diluted Pseudomonas-CF-IgG antigen to each well of a Maxisorp ELISA plate (NUNC®).
3. Incubate for 1 hour at room temperature.
4. Aspirate and wash 3 times (3 min. soak/wash) with washing buffer.
5. Add 100 µL dilution buffer to each well and incubate overnight at 4°C or 1 hour at room temperature.
6. Aspirate and wash 2 times (3 min. soak/wash) with washing buffer.
7. Pseudomonas-CF-IgG standard serum is diluted in dilution buffer; 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16,000, 1:32,000, 1:64,000. Add 100 µL of the diluted standard serum to each defined standard well.
8. Patient serum is diluted 1:100 in dilution buffer. Add 100 µL of the diluted patient serum to the defined well.
9. Incubate for 1 h at room temperature.
10. Aspirate and wash 3 times (3 min. soak/wash) with washing buffer.
11. Rabbit-Anti-Human IgG HRP (DAKO® P0214) is diluted 1:20,000 in dilution buffer. Add 100 µL of the dilution to each well.
12. Incubate for 1 hour at room temperature.
13. Aspirate and wash 5 times (3 min. soak/wash) with washing buffer.
14. Add 100 µL TMB Plus Standard to each well.
15. Incubate at room temperature for 1 hour in the dark.
16. Add 100 µL H₂SO₄ (2N) to each well (ends the reaction).
17. Read the absorbance of the solution in the wells within 10 minutes using an ELISA reader set to 450 nm.

The absorbance of the standard serum dilutions are used to construct a standard curve. The absorbance of the patient sample is extrapolated on the standard curve and divided by 10 (dilution factor) to calculate ELISA Units. The mean normal absorbance, of sera from non-infected Danish CF patients is 0.66, SD ± 0.82.

Support

Reference laboratory at the Department of Clinical Microbiology & Danish CF Centre, Rigshospitalet, University of Copenhagen, Denmark. Sera producing unexplainable results may be sent to the reference laboratory together with information about the bacteriological status of the patient for absorption of possible cross-reactive antibodies. E-mail: hoiby@inet.uni2.dk for further information.

Storage and shelf life

Store the sealed vial of lyophilized *Pseudomonas*-CF-IgG antigen at room temperature. Expiry date of the sealed vial is printed on the package. The *Pseudomonas*-CF-IgG antigen undiluted stock solution can be frozen at -20°C and thaw at room temperature and shortly after refrozen for at least 20 times without any change of activity. Store the *Pseudomonas*-CF-IgG standard antiserum at -20°C. Expiry date of the sealed vial is printed on the package. The 100 µL standard antiserum may be repeatedly thaw and refrozen until empty without any change of activity. It should be used to calibrate a locally obtained standard antiserum.

References

- 1) Høiby, N., Collins, M.T., Espersen, F., Hertz, J.B., Hoff, G.E., Schiøtz, P.O.: Taxonomic application of crossed immunoelectrophoresis. *Internat. J. Syst. Bacteriol.* 37:229-240; 1987.
- 2) Pedersen, S.S., Espersen, F., Høiby, N.: Diagnosis of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 25:1830-1836; 1987.
- 3) Pressler, T., Pedersen, S.S., Espersen, F., Høiby, N., Koch, C.: IgG subclass antibodies to *Pseudomonas aeruginosa* in sera from patients with chronic Ps. *aeruginosa* infection investigated by ELISA. *Clin. exp. Immunol.* 81:428-434; 1990.
- 4) Valerius, N.H., Koch, C. & Høiby, N.: "Prevention of chronic colonization with *Pseudomonas aeruginosa* in patients with Cystic Fibrosis by early treatment with Ciprofloxacin and inhalation with Colistin". *Lancet* 338: (1991):725-26; 1991.
- 5) Frederiksen, B., Lanng, S., Koch, C., Høiby, N.: Improved survival in the Danish cystic fibrosis centre - results of aggressive treatment. *Pediatr. Pulmonol.* 21:153-158, 1996.
- 6) Frederiksen, Koch, C. & Høiby, N.: Antibiotic treatment at time of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration in pulmonary function in patients with cystic fibrosis. *Pediatr. Pulmonol.* 23:330-335, 1997.
- 7) Frederiksen, B., Koch, C., Høiby, N.: Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974- 1995). *Pediatr. Pulmonol.* 28:159-66;1999
- 8) Döring, G., Høiby, N. for the consensus study group: Early intervention and prevention of lung disease in cystic fibrosis: a European consensus. *J. Cystic Fibrosis* 3:67-91; 2004.
- 9) Høiby, N., Frederiksen, B., Pressler, T.: Eradication of early *Pseudomonas aeruginosa* infection. *J. Cystic Fibrosis* 4:49-54; 2005.



3rd Edition, July 2008 · 61290