

PSEUDOMONAS-CF-IgG

ELISA KIT



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*prevents and controls
infectious diseases,
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congenital disorders*



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PSEUDOMONAS-CF-IgG ELISA Kit is for the quantitative measurement of the antibody level to *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.

The test result should not stand alone but be accompanied by results from culturing samples for *P. aeruginosa*.

Description

The PSEUDOMONAS-CF-IgG ELISA Kit contains:

- 1 Maxisorp ELISA plate (NUNC®) (store at RT)
- 9 mg lyophilized antigen (store at RT)
(sonication of *P. aeruginosa* serotypes O-1 through O-17)
- 1 vial pooled human standard antiserum (store at RT)
(antibodies against *P. aeruginosa*)
- 0.5 mL Sterile distilled water (store at RT)
- 12 mL Coating buffer (store at RT)
- 250 mL Washing buffer (store at RT)
- 250 mL Dilution buffer (store at RT)
- 0.1 mL Rabbit-Anti-Human IgG HRP (store at 2-8°C)
- 12 mL Sulphuric acid (2M) (store at RT)
- 12 mL TMB Plus Substrate (store at 2-8°C)

Equipment required

ELISA reader set at 450 nm.

Principle

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

Limitations

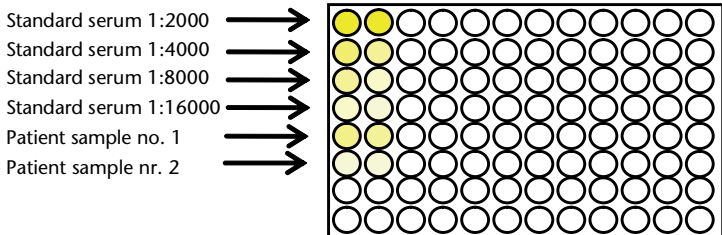
Non-specific antibodies due to cross-reactivity between *P. aeruginosa* and other bacterial species are low and correlates with taxonomic relatedness.

Procedure

1. ELISA procedure

The human standard antiserum and unknown samples should be assayed in duplicates.

Example of a setup.



To make the best possible use of the ELISA Kit we recommend to perform at least 4 patient samples at the same time.

2. Preparation of dilutions

Solution A: Antigen solution for coating ELISA plate (use in step 1)

- Add 100 μL sterile distilled water to the vial containing 9 mg Pseudomonas-CF-IgG antigen, and resolve the lyophilized antigen.
- Dilute the amount to be used 1:2000 in coating buffer.
- Dilute only the amount to be used the same day.
- Store the remaining undiluted antigen stock at -20°C .
The stock solution can be frozen and unfrozen up to 20 times.

Solution B: Dilution of human standard antiserum (use in step 3)

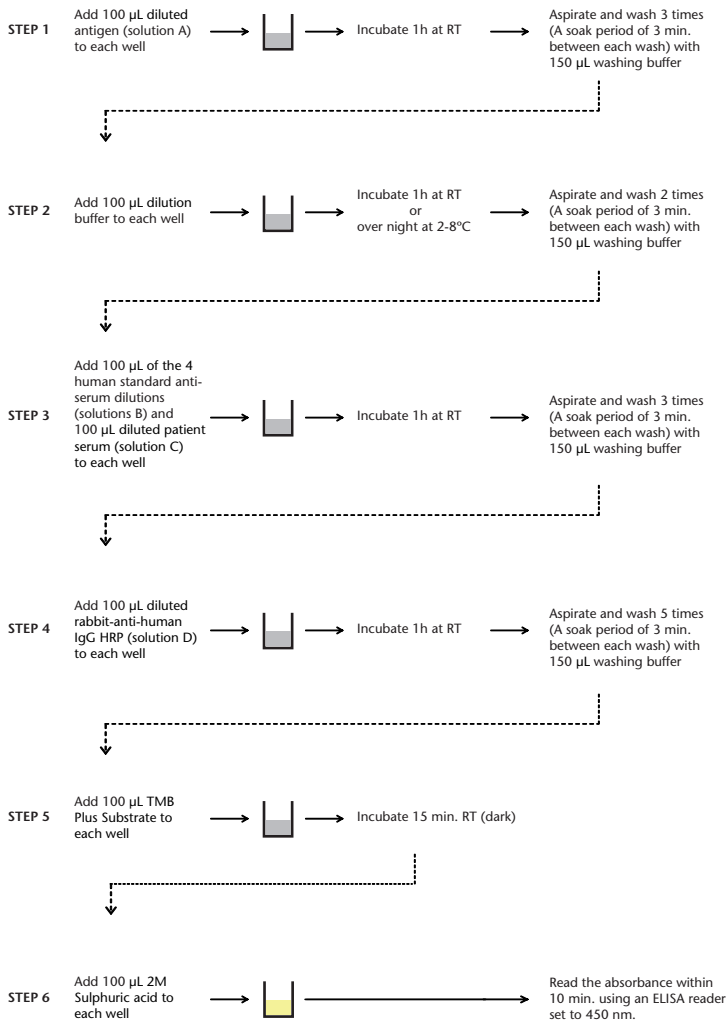
- Add 100 μL sterile distilled water to the vial with human standard antiserum, and resolve the antiserum.
- Dilute the human standard antiserum 1:2,000, 1:4,000, 1:8,000 and 1:16,000 in dilution buffer.
- Dilute only the amount to be used the same day.
- Store the remaining undiluted human standard antiserum at -20°C .
- The human standard antiserum may be repeatedly unfrozen and refrozen until empty without any change of activity.

Solution C: Dilution of patient antiserum (use in step 3)

- The patient antiserum has to be diluted 1:100 in dilution buffer (ex. 10 μL antiserum added to 990 μL dilution buffer).
- Dilute only the samples to be measured the same day.

Solution D: Dilution of Rabbit-Anti-Human IgG HRP (use in step 4)

- The Rabbit-Anti-Human IgG HRP has to be diluted 1:20,000 in dilution buffer (ex. 1 μL Rabbit-Anti-Human IgG HRP is added to 20 mL dilution buffer). Mix thoroughly.
- Dilute only the amount to be used the same day.

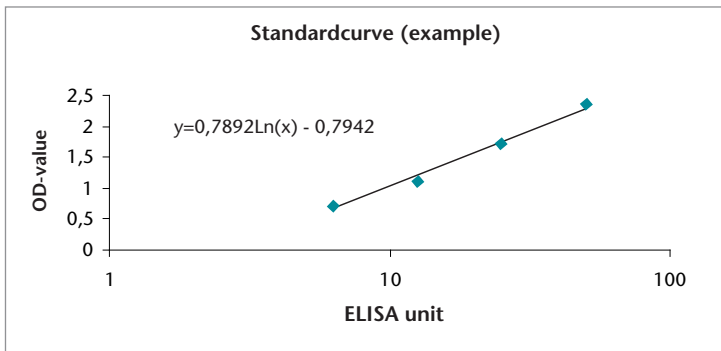


Calculation of results

The absorbance of the human standard antiserum dilutions are used to construct a standard curve. The following illustrates an example of data calculation. The human standard antiserum sample 1:1000 equals 100 ELISA units. Calculation of the ELISA units for the dilutions 1:2000 to 1:16000 are as follows:

Dilution factor of human standard serum	ELISA unit	OD ₄₅₀ -value
1:2000	50.00	2.34
1:4000	25.00	1.71
1:8000	12.50	1.13
1:16000	6.25	0.71

Draw the standard curve as shown below with the OD₄₅₀-value as a function of the ELISA unit (the x-axis should be logarithmic). The regression line of the points must be linear and the equation should be shown.



The OD₄₅₀-values of the patient samples should be converted to ELISA units using the equation for the standard curve.

$$OD_{450}\text{-value} = 0.7892\text{Ln}(\text{ELISA unit}) - 0.7942$$



$$\text{ELISA unit} = \text{eksp}((OD_{450}\text{-value} + 0.7942) / 0.7892)$$

Since the patient sample was diluted 10 times less than the human standard antiserum the ELISA unit result should be divided by 10.

Patient	OD ₄₅₀ -value of patient samples	ELISA unit	ELISA unit/10
1	2.60	73.96	7.396
2	0.53	5.35	0.535
3	1.96	32.78	3.278
4	0.89	8.45	0.845

If the patient sample has to be diluted more than 1:100 to fit the standard curve the ELISA unit has to be divided by 1000/the new dilution factor to give the correct result (ex. the sample is diluted 1:300 the ELISA unit result is divided by 3,33 (=1000/300)).

The cut-off value for a positive ELISA unit/10 result is 2.96 (see explanation below) which means that patient number 1 and 3 are positive and patient number 2 and 4 are negative.

Interpretation of results

The normal ELISA unit value of non-infected persons of *P. aeruginosa* IgG is 0.66 +/- 1.64 (mean +/- 2 times standard deviation). The 95% upper normal limit is therefore 2.30 and a significant increased titer compared to normal controls is > 2.30.

The normal ELISA unit value of non-*P. aeruginosa* infected CF patient of *P. aeruginosa* IgG is 0.57 +/- 2.39 (mean +/- 2 times standard deviation). The 95% upper normal limit is therefore 2.96 and a significant increased titer compared to non-*P. aeruginosa* infected CF patients is = or > 2.96.

The difference between non-infected persons and non-*P. aeruginosa* infected CF patients is due to cross-reactive antibodies induced by e.g. *H. influenzae* infections.

	Culture positive for <i>P. aeruginosa</i>	Culture negative for <i>P. aeruginosa</i>
IgG Elisa unit > 2.96	A	B
IgG Elisa unit = or < 2.96	C	D

Group A: Probably chronic *P. aeruginosa* infection

A positive culture of *P. aeruginosa* accompanied by increased titer of IgG above upper normal limit of non-*P. aeruginosa* infected CF patients (> 2.96) indicate chronic *P. aeruginosa* infection especially if the strain is mucoid.

Group B: Probably not chronic *P. aeruginosa* infection, but repeat culture
Negative *P. aeruginosa* cultures and increased IgG titers (> 2.96) requires repeated cultures to rule out chronic infection. IgG titers may only decrease slowly after eradication of *P. aeruginosa*.

Group C: Probably intermittent *P. aeruginosa* colonization

Positive cultures for *P. aeruginosa* accompanied with rising IgG titers even below the upper normal limit of non-*P. aeruginosa* infected CF patients (=/ $<$ 2.96) is an indication of onset of chronic infection now or during the next year, whereas low stable titers indicate intermittent colonization.

Group D: Probably not *P. aeruginosa* colonization or chronic infection

Using series of measurements of IgG titers over time in individual patients may show rise of titers even below the upper normal limits (=/ $<$ 2.96), which require individual judgements by the clinicians taking into consideration the results of repeated cultures for *P. aeruginosa*.

Support

Reference laboratory at the Department of Clinical Microbiology & Danish CF Centre, Rigshospitalet, University of Copenhagen, Denmark. Sera producing unexplainable results may be send to the reference laboratory together with information about the bacteriological status of the patient for

absorption of possible cross-reactive antibodies. E-mail: hoiby@hoibyniels.dk for further information.

Storage and shelf life

After recipient of the PSEUDOMONAS-CF-IgG ELISA Kit the vial with pooled human standard antiserum has to be stored at - 20°C. The Rabbit-Anti-human IgG HRP and the TMB Plus Standard should be stored at 2-8°C. The other reagents can be stored at room temperature.

The antigen stock solution should be stored at - 20°C and can be unfreezed at room temperature and shortly after refrozen for at least 20 times without any change of activity.

The human standard antiserum is stored at - 20°C and may be repeatedly freezed and unfreezed until empty without any changes of activity.

References

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