SSI DIAGNOSTICA

Instruction For Use

PSEUDOMONAS-CF-IGG

ELISA KIT

ANTIGEN

CONTROL ANTISERUM

ENGLISH

GENERAL INFORMATION

Quality Certificate

SSI Diagnostica is quality assured and certified in accordance with ISO 13485. Certificate of analysis can be downloaded from our website www.ssidiagnostica.com.

Support

Reference laboratory at the Department of Clinical Microbiology & Danish CF Centre, Rigshospitalet, University of Copenhagen, Denmark. Antisera 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@hoibyniels.dk for further information.

Literature about development of the ELISA kit and surveillance of CF patients infected with *P. aeruginosa* can be found under references[1-10].

Additional information about the Pseudomonas-CF-IgG ELISA Kit, Antigen Pool and Control Antiserum is available at our homepage www.ssidiagnostica.com.
If you have any difficulties using the products, please contact SSI Diagnostica at info@ssidiagnostica.com.

ABBREVIATION LIST

CF	Cystic Fibrosis
ELISA	Enzyme Linked Immunosorbent Assay
H. influenzae	*Haemophilus influenzae*
HRP	Horseradish Peroxidase
IgG	Immunoglobolin G
P. aeruginosa	*Pseudomonas aeruginosa*

PSEUDOMONAS-CF-IGG

ELISA KIT
ANTIGEN POOL
CONTROL ANTISERUM

Intended use

The Pseudomonas-CF-IgG ELISA method is for the quantitative measurement of the antibody level to *P. aeruginosa* in human antiserum samples.

Description

The Pseudomonas-CF-IgG ELISA Kit contains all the below materials to perform the ELISA test:

- 1 Maxisorp ELISA plate (NUNC®) **(store at 15-25°C**)
- 9 mg Pseudomonas-CF-IgG Antigen, lyophilized **(store at 15-25°C)**
  (sonicated *P. aeruginosa* serotypes O-1 through O-17)
- 0.1 mL Pseudomonas-CF-IgG Control Antiserum **(store at -20°C)**
  (pooled human antibodies against *P. aeruginosa*)
- 0.5 mL sterile distilled water **(store at 15-25°C)**
- 12 mL Coating Buffer **(store at 15-25°C)**
- 2 x 250 mL Washing Buffer **(store at 15-25°C)**
- 250 mL Dilution Buffer **(store at 15-25°C)**
- 0.1 mL Rabbit-anti-human IgG HRP **(store at 2-8°C)**
- 12 mL Sulphuric Acid (2M) **(store at 15-25°C)**
- 12 mL TMB Plus 2 substrate **(store at 2-8°C)**

With the above materials provided it is possible to perform up to 96 reactions.

Materials and Instruments Required but not Provided

- Pipettes
- ELISA reader set at 450 nm

If only the 9 mg Pseudomonas-CF-IgG antigen and the 0.1 mL Pseudomonas-CF-IgG control antiserum have been purchased the following materials are needed:

Materials and Instruments Required but not Provided

- Maxisorp ELISA plates (NUNC®) (**store at 15-25°C**)
- Sterile distilled water **(store at 15-25°C)**
- Coating Buffer **(store at 15-25°C**)
  (1.724 g NaH_2PO_4, H_2O + 13.40 g Na_2HPO_4, 12 H_2O + 42.35 g NaCl + 5 L H_2O)
- Washing Buffer **(store at 15-25°C)**
  (2 L Coating Buffer + 2 mL Tween 20)
- Dilution Buffer **(store at 15-25°C)**
  (1 L Coating Buffer + 1 mL Tween 20 + 15 g NaCl)
- Rabbit-anti-human IgG HRP (DAKO® P0214) **(store at 2-8°C)**
- Sulphuric acid (2M) (store at 15-25°C)
- TMB Plus 2 substrate (Kem-En-Tec Diagnostics) **(store at 2-8°C)**
- Pipettes
- Glass ware
- Balance
- ELISA reader set at 450 nm

Principle

The Pseudomonas-CF-IgG ELISA method has a traditional ELISA setup. More than 64 different antigens are detectable in the antigen pool. The results from the pooled human standard antiserum (Pseudomonas-CF-IgG Control Antiserum) are used to calculate the concentration of antibody against *P. aeruginosa* in the patient sample.

Chronic *P. aeruginosa* infections can reliably be discriminated from intermittent colonization by measuring IgG 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*.

Precautions

False positive results due to cross-reactivity between *P. aeruginosa* and related Gram-negative bacterial species (e.g. Burkholderia) are possible but not very common.

Procedure

Pseudomonas-CF-IgG Control Antiserum and unknown samples should be assayed in duplicates.

Example of a set-up

To make the best possible use of the ELISA Kit we recommend performing at least four patient samples at the same time. Figure 1 shows an example of a set-up in an ELISA plate.

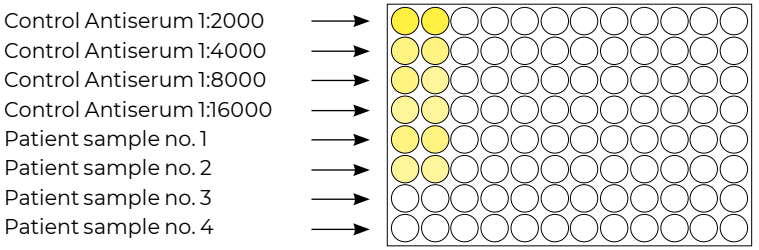

Figure 1. Example of a set-up in an ELISA plate.

Prepare solution A, B, C and D as described below.

Solution A:

Dilution of Pseudomonas-CF-IgG antigen (used in step 1, see figure 2)

1. Add 100 µL sterile distilled water to the vial containing 9 mg Pseudomonas-CF-IgG antigen and resolve the lyophilized antigen.
2. Dilute the amount to be used 1:2000 in Coating Buffer.
3. Dilute only the amount to be used the same day.
4. The stock of Pseudomonas-CF-IgG antigen solution can be repeatedly frozen and thawed 20 times without change of activity.

Solution B:

Dilution of Pseudomonas-CF-IgG Control Serum (used in step 3, see figure 2)

1. Dilute the Pseudomonas-CF-IgG Control Antiserum 1:2000, 1:4000, 1:8000 and 1:16000 in Dilution Buffer.
2. Dilute only the amount to be used the same day.
3. Store the remaining undiluted Pseudomonas-CF-IgG Control Antiserum at -20°C.
4. The undiluted Pseudomonas-CF-IgG Control Antiserum can be repeatedly frozen and thawed until the vial is empty without change of activity.

Solution C:

Dilution of patient antiserum (used in step 3, see figure 2)

1. The patient antiserum must be diluted 1:100 in Dilution Buffer (ex. 10 µL antiserum added to 990 µL Dilution Buffer).
2. Dilute only the samples to be measured the same day.

Solution D:

Dilution of Rabbit-anti-human IgG HRP (used in step 4, see figure 2)

The Rabbit-anti-human IgG HRP must be diluted 1:20000 as described below.

1. Add 5 µL Rabbit-anti-human IgG HRP to 995 µL dilution buffer (1:200).
2. Add 150 µL of the 1:200 dilution to 14850 µL Dilution Buffer.
3. Mix thoroughly.
4. Dilute only the amount to be used the same day.

Perform all steps in the procedure as described in figure 2.

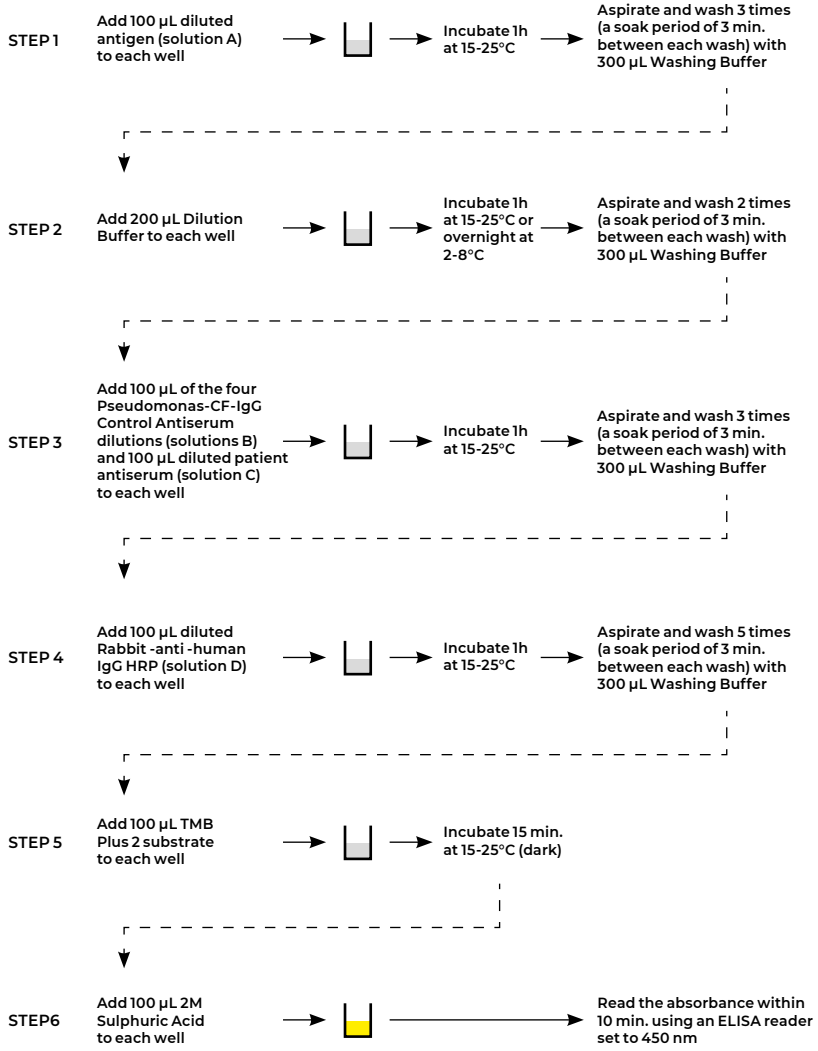

Figure 2. The ELISA procedure.

Calculation of the Analysis Results

The absorbance of the diluted Pseudomonas-CF-IgG Control Antiserum (the human standard antiserum) is 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 shown in table 1.

Table 1. Calculated ELISA units for the dilutions 1:2000 to 1:16000.

DILUTION FACTOR OF HUMAN STANDARD ANTISERUM	ELISA UNIT	OD_{450}-VALUE
1:2000	50.00	2.34
1:4000	25.00	1.71
1:4000	12.50	1.13
1:8000	6.25	0.71

Draw the standard curve as shown in figure 3 with the OD_{450}-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.

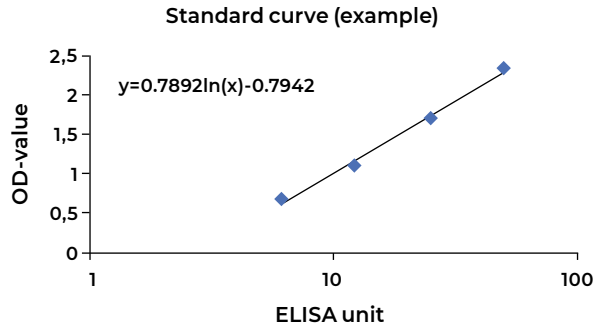

Figure 3. An example of the correlation between ELISA units and the OD_{450}-value.

The OD_{450}-values of the patient samples should be converted to ELISA units using the equation for the standard curve.

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

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

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

Table 2. Calculation of ELISA units in the patient samples.

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 must be diluted more than 1:100 to fit the standard curve the ELISA unit must be divided by 1000/the new dilution factor to give the correct result (ex. the patient 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 the Analysis 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 significantly increased titer compared to normal controls is > 2.30.

The normal ELISA unit value of non-*P. aeruginosa* infected CF patients 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 significantly increased titer compared to non-*P. aeruginosa* infected CF patients is > 2.96 (group A, B, C and D are described in table 3).

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.

Table 3. Interpretation of the IgG ELISA unit value compared to *P. aeruginosa* culture results. The conclusions on group A, B, C and D are described below the table.

	Culture positive for *P. aeruginosa*	Culture negative for *P. aeruginosa*
IgG ELISA unit > 2.96	**A**	**B**
IgG ELISA unit ≤ 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) indicates chronic *P. aeruginosa* infection especially if the strain is mucoid.

Group B: Probably not chronic *P. aeruginosa* infection, but repeat the culture
Negative *P. aeruginosa* cultures and increased IgG titers (>2.96) require 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*.

Storage and Shelf Life

After receiving the Pseudomonas-CF-IgG ELISA Kit, the Pseudomonas-CF-IgG Antigen or the Pseudomonas-CF-IgG Control Antiserum store the materials as follows:

- the stock solution of Pseudomonas-CF-IgG Antigen must be stored at -20°C. It can be subjected to 20 freeze-thaw cycles without any change of activity.
- the undiluted Pseudomonas-CF-IgG Control Antiserum should immediately be stored at -20°C. It can be subjected to freeze-thaw cycles until the vial is empty without any change of activity.
- the Rabbit-anti-human IgG HRP should be stored at 2-8°C.
- the TMB Plus 2 Substrate should be stored at 2-8°C.
- all other reagents can be stored at 15-25°C.

References

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Information and ordering

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