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 -20ºC)* *(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 – 8ºC)*
12 mL Sulphuric acid (2M) *(store at RT)*
12 mL TMB Plus 2 Substrate *(store at 2 – 8ºC)*

**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.

Standard serum 1:2000
Standard serum 1:4000
Standard serum 1:8000
Standard serum 1:16000
Patient sample no. 1
Patient sample nr. 2

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.
- The stock solution can be repeatedly frozen and thawed 20 times without change of activity.

Solution B: Dilution of human standard antiserum (use in step 3)
- 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 -20ºC.
- The human standard antiserum can be repeatedly frozen and thawed until the vial is empty without 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 in two steps:
  Step 1: 5 µL rabbit-anti-human IgG HRP is added to 995 µL dilution buffer (1:200).
  Step 2: and then 150 µL of the 1:200 dilution is added to 14,850 µL dilution buffer. Mix thoroughly.
- Dilute only the amount to be used the same day.
**STEP 1**
Add 100 µL diluted antigen (solution A) to each well → Incubate 1h at RT → Aspirate and wash 3 times (A soak period of 3 min. between each wash) with 300 µL washing buffer

**STEP 2**
Add 200 µL dilution buffer to each well → Incubate 1h at RT or over night at 2 - 8ºC → Aspirate and wash 2 times (A soak period of 3 min. between each wash) with 300 µL washing buffer

**STEP 3**
Add 100 µL of the 4 human standard anti-serum dilutions (solutions B) and 100 µL diluted patient serum (solution C) to each well → Incubate 1h at RT → Aspirate and wash 3 times (A soak period of 3 min. between each wash) with 300 µL washing buffer

**STEP 4**
Add 100 µL diluted rabbit-anti-human IgG HRP (solution D) to each well → Incubate 1h at RT → Aspirate and wash 5 times (A soak period of 3 min. between each wash) with 300 µL washing buffer

**STEP 5**
Add 100 µL TMB Plus 2 Substrate to each well → Incubate 15 min. RT [dark] → Read the absorbance within 10 min. using an ELISA reader set to 450 nm.

**STEP 6**
Add 100 µL 2M Sulphuric acid to each well
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:

<table>
<thead>
<tr>
<th>Dilution factor of human standard serum</th>
<th>ELISA unit</th>
<th>OD(_{450}) -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2000</td>
<td>50.00</td>
<td>2.34</td>
</tr>
<tr>
<td>1:4000</td>
<td>25.00</td>
<td>1.71</td>
</tr>
<tr>
<td>1:8000</td>
<td>12.50</td>
<td>1.13</td>
</tr>
<tr>
<td>1:16000</td>
<td>6.25</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Draw the standard curve as shown below 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.

The OD\(_{450}\)-values of the patient samples should be converted to ELISA units using the equation for the standard curve.
\[
\text{OD}_{450} \text{-value} = 0.7892 \ln(\text{ELISA unit}) - 0.7942
\]

\[
\text{ELISA unit} = \exp\left(\frac{\text{OD}_{450} \text{-value} + 0.7942}{0.7892}\right)
\]

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>(\text{OD}_{450} \text{-value of patient samples})</th>
<th>ELISA unit</th>
<th>ELISA unit/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.60</td>
<td>73.96</td>
<td>7.396</td>
</tr>
<tr>
<td>2</td>
<td>0.53</td>
<td>5.35</td>
<td>0.535</td>
</tr>
<tr>
<td>3</td>
<td>1.96</td>
<td>32.78</td>
<td>3.278</td>
</tr>
<tr>
<td>4</td>
<td>0.89</td>
<td>8.45</td>
<td>0.845</td>
</tr>
</tbody>
</table>

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-\( P. \text{aeruginosa} \) infected CF patient of \( P. \text{aeruginosa} \) 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-\( P. \text{aeruginosa} \) infected CF patients is = or > 2.96.

The difference between non-infected persons and non-\( P. \text{aeruginosa} \) infected CF patients is due to cross-reactive antibodies induced by e.g. \( H. \text{influenzae} \) infections.

<table>
<thead>
<tr>
<th>Culture positive for ( P. \text{aeruginosa} )</th>
<th>Culture negative for ( P. \text{aeruginosa} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG Elisa unit &gt; 2.96</td>
<td>A</td>
</tr>
<tr>
<td>IgG Elisa unit = or &lt; 2.96</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>D</td>
</tr>
</tbody>
</table>

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

**Group B: Probably not chronic \( P. \text{aeruginosa} \) infection, but repeat culture**
Negative \( P. \text{aeruginosa} \) cultures and increased IgG titers (> 2.96) requires repeated cultures to rule out chronic infection. IgG titers may only decrease slowly after eradication of \( P. \text{aeruginosa} \).
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 receiving the PSEUDOMONAS CF-IgG ELISA Kit the human standard antiserum should immediately be stored at -20 °C. The Rabbit-Anti-Human IgG HRP and TMB Plus 2 Substrate are stored at 2-8 °C. The other reagents can be stored at room temperature.

The diluted lyophilized antigen is stored at -20 °C. It can be subjected to 20 freeze-thaw cycles without any change of
activity. The human standard antiserum is stored at -20°C. It can be subjected to freeze-thaw cycles until the vial is empty without any change of activity. The antigen and antiserum is thawed at room temperature and refrozen immediately after use.

References

**Information and ordering**
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