anti-Pseudomonas aeruginosa Antibody
ELISA E15

*Pseudomonas aeruginosa*, a Gram-negative bacterium ubiquitously distributed in moist environment, causes about 10% of all nosocomial infections. This opportunistic pathogen leads to acute and chronic types of infection within various organs of susceptible patient groups, e.g. in patients with cystic fibrosis (CF). Early detection of infection and therewith early therapy improve the outcome significantly.

*P. aeruginosa* infection provokes a rapid production of antibodies to a large number of *P. aeruginosa* antigens in CF patients.

Mediagnost sensitive antibody detection system detects a *P. aeruginosa* infection very early at the onset, *when microbiological information is not yet available*. The assay allows the commencement of anti-Pseudomonas chemotherapy early after onset of the infection and also serves as a tool to control the efficiency of antibiotic therapy ([Ratjen F et al.:Lancet. 2001 Sep 22;358(9286):983-4]). Due to the use of three *P. aeruginosa* antigens which are highly immunogenic and present in different parts from nearly all *P. aeruginosa* strains, this test has an extremely high sensitivity and predictive value ([Kappler, M et al.: Thorax. 2006 61(8):614-618]). Most patients immune systems react with at least one of the three antigens and therefore false-negative results are almost impossible, as well as false-positive.

Based on antibody titers the following diagnostic interpretation of test results is used in practice:

<table>
<thead>
<tr>
<th>Title</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1:500</td>
<td>negative</td>
</tr>
<tr>
<td>1:500 to 1:1.250</td>
<td>border-line</td>
</tr>
<tr>
<td>&gt; 1:1.250</td>
<td>positive</td>
</tr>
</tbody>
</table>

**Mediagnost Anti-Pseudomonas aeruginosa ELISA E15**

This sandwich enzyme immunoassay detects antibodies against *Pseudomonas aeruginosa* antigens. Serum or plasma samples are diluted and added to the wells of a microtitre plate, which has been previously coated with the *Pseudomonas aeruginosa* antigens alkaline protease, elastase or exotoxin A. Specific antibodies in the sample bind to the antigens, by Peroxidase-coupled anti-human IgG antibody bound antibodies are detected.

**Assay Features E15**

- Easy sample winning
- Inter-assay variance of 6.55%
- Intra-assay variance of 4.73%
- In comparison to pharyngeal swab 100% same results were obtained (104 samples)
- Control sera included

Mediagnost anti-Pseudomonas aeruginosa ELISA E15

Dilution of Reagents and Samples

<table>
<thead>
<tr>
<th>Conjugate concentrate KK</th>
<th>in Dilution Buffer VP</th>
<th>1:100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing buffer WP</td>
<td>in Aqua dest. (e.g. add 100 ml WP in to a graduated flask and fill with A.dest to 2000 ml)</td>
<td>1:20</td>
</tr>
</tbody>
</table>

Dilute samples 1:1000 with dilution buffer (qualitative test). For quantitative antibody assays it is recommended to perform dilutions of 1:1,000, 1:10,000 and 1:100,000.

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Reagents</th>
<th>Plate 1AP</th>
<th>Plate 2Ela</th>
<th>Plate 3Exo</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 100 µl</td>
<td>Negative Control NK</td>
<td>A1/A2</td>
<td>A1/A2</td>
<td>A1/A2</td>
</tr>
<tr>
<td>2 x 100 µl</td>
<td>Control Serum KS</td>
<td>C1/C2</td>
<td>C1/C2</td>
<td>C1/C2</td>
</tr>
<tr>
<td>2 x 100 µl</td>
<td>Sample dilution</td>
<td>Pipette in the rest of the wells according to requirements</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Seal the wells with the seeling tape

Incubation: 2 h at 37°C

3 x 300 µl | Aspirate the contents of the wells and wash 3x with 300 µl each Wash Buffer WP/ well | in each well |
|-----------|---------------------------------|--------------|

100 µl | 1:100 diluted Conjugate concentrate KK | in each well |

Seal the wells with the seeling tape

Incubation: 2 h at 37°C

3 x 300 µl | Aspirate the contents of the wells and wash 3x with 300 µl each Wash Buffer WP/ well | in each well |
|-----------|---------------------------------|--------------|

100 µl | Substrate Solution S | in each well |

Incubation: 30 min in the dark at RT

100 µl | Stopping Solution SL | in each well |

Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.

Literature

Brett M M, Ghoneim A T M, Littlewood J M: Serum antibodies to Pseudomonas aeruginosa in cystic fibrosis. Archives of Diseases in Childhood, 1986, 61, 1114-20


Winnie GB, Cowan RG. Respiratory tract colonization with Pseudomonas aeruginosa in cystic fibrosis: correlation between anti-Pseudomonas aeruginosa antibody levels and pulmonary function. Pediatr Pulmonol 1991;10:92-100