Anti-Acetylcholine Receptor ELISA (IgG)

**Indication**: Test system for the in vitro determination of autoantibodies against acetylcholine receptor (AChR) in human serum or plasma for the diagnosis of the following disease: myasthenia gravis (MG).

**Clinical significance**: In MG autoantibodies against AChR in the motor end plate cause disturbances in neuromuscular transmission. This can lead to progressive weakness of vital muscles and lead to death. If diagnosed at an early stage, life-saving treatment is possible. MG is divided into different forms. These encompass acquired forms of MG, either generalised MG or MG with exclusively ocular syndromes, and genetic forms.

The prevalence of MG is estimated to be 40 to 125 cases per million people in Europe and 50 to 200 in America (USA). MG can occur from early childhood to old age. Women, particularly young women of 20 to 30 years of age, and men older than 50 years of age are more likely than others to be afflicted with the disease.

Various serological tests are available for MG diagnostics. Initially, an acetylcholine inhibitor test can be performed. If this shows normal values, autoantibodies against AChR are investigated. The Anti-Acetylcholine Receptor ELISA and the Anti-Acetylcholine Receptor RIA are highly specific and sensitive methods for diagnosis and monitoring and provide the best and most reliable results.

Almost 90% of patients with acquired generalised MG exhibit autoantibodies against AChR. In MG patients with exclusively ocular symptoms they are detected in approximately 60%. Autoantibodies against AChR are not present in patients with genetic forms of MG (approximately 5 to 10% of all MG cases). Since the absence of autoantibodies against AChR does not exclude MG completely, electromyography (EMG) should be used in suspected cases for clarification. Autoantibodies against AChR rarely occur without clinical symptoms of MG, but if they do, they indicate a high risk for the disease. These cases are often also associated with other autoimmune diseases. The course of MG can be monitored and assessed by regular investigation of anti-AChR autoantibody titers.

**Application of the Anti-Acetylcholine Receptor ELISA (IgG)**: The EUROIMMUN enzyme immunoassay based on fully recombinant acetylcholine receptors with a γ or ε subunit is a sensitive and specific test for serological diagnosis of myasthenia gravis. The quantity and mixing ratio of purified adult and foetal receptors were selected so as to provide the test with a large linear dilution range.
Test characteristics
Anti-Acetylcholine Receptor ELISA (IgG)

Linearity: Positive patient sera show a linear range when diluted with normal serum. Above this linear range, an increase in the autoantibody concentration of the samples leads to a non-proportional plateau effect. Quantitative determination of acetylcholine receptor antibodies is only meaningful in the linear range of 0.25 to approx. 6.0 nmol/l. Consequently, measurements above the linear range give false values. Both the linear range and the plateau range differ from patient serum to patient serum. Therefore, both ranges must be determined for each positive serum using different dilutions. During patient follow-up, the appropriate dilution factor can be estimated from preceding values. Based on current experience, the range from 0.25 to approx. 6.0 nmol/l is linear.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay and inter-assay CVs are each based on 15 determinations.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Intra-assay variation, n= 1 x 15</th>
<th>Inter-assay variation, n= 15 x 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value (nmol/l)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>1</td>
<td>0.254</td>
<td>13.2</td>
</tr>
<tr>
<td>2</td>
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<td>7.1</td>
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<tr>
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<td>6.6</td>
</tr>
<tr>
<td>4</td>
<td>5.349</td>
<td>6.1</td>
</tr>
</tbody>
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Sensitivity and specificity: The levels of anti-acetylcholine receptor antibodies were measured in 90 sera using the EUROMMUN Anti-Acetylcholine Receptor ELISA (IgG) and the Anti-AChR RIA. With respect to the radioimmunoassay the sensitivity of the ELISA was 96% and the specificity 97% (borderline sera excluded).

Reference range: 151 healthy blood donors showed anti-acetylcholine receptor antibody concentrations of up to 0.86 nmol/l. The mean was 0.18 nmol/l with a standard deviation of 0.18 nmol/l. The 95th percentile was determined as 0.449 nmol/l. 95% of healthy blood donors were anti-acetylcholine receptor negative.

Technical data:

Antigen: Recombinant human foetal and adult acetylcholine receptors from HEK cells

Calibration: Quantitative, in nanomol per litre (nmol/l).
Calibrator 1 0 nmol/l
Calibrator 2 0.25 nmol/l
Calibrator 3 0.75 nmol/l
Calibrator 4 2.5 nmol/l
Calibrator 5 8 nmol/l

Result interpretation: EUROMMUN recommends interpreting results as follows:
- <0.40 nmol/l negative
- ≥0.40 bis <0.50 nmol/l borderline
- ≥0.50 nmol/l positive
The cut-off should be 0.5 nmol/l.

Sample dilution: Serum or plasma; 1:26 in sample dilution buffer.

Reagents: Ready-for-use, with the exception of the wash buffer (10 x).

Test procedure: 90 min (sample incubation at 37°C)/60 min (anti-human IgG incubation at 37°C)/15 min (substrate incubation at room temperature).

Measurement: 450 nm. Reference wavelength between 620 nm and 650 nm.

Test kit format: 96 break-off wells. Kit includes all necessary reagents.

Order number: EA 1435-9601 G