Indication: Test system for the in vitro determination of antibodies against dsDNA in human serum or plasma for the diagnosis of the following disease: systemic lupus erythematosus (SLE).

Clinical significance: Antibodies against DNA are distinguished into two different types: Antibodies against double-stranded, native DNA (dsDNA) and antibodies against single-stranded, denatured DNA. Antibodies against dsDNA react mainly with epitopes in the deoxyribose phosphate backbone of the double helix. On the other hand, antibodies defined as reactive with ssDNA recognize epitopes of purine and pyrimidine bases but they may also react with epitopes of the deoxyribose phosphate backbone. Antibodies against dsDNA are the main focus in the serological diagnosis of systemic lupus erythematosus (SLE). These antibodies can be found in 60% to 90% of patients, depending on the activity of the disease. Antibodies against nucleosomes are also an exclusive marker of SLE, provided that they are determined using a perfect test system whose target antigen must be free of histone H1, Scl-70 and other non-histone proteins.

Application of the Anti-dsDNA-NcX ELISA: Using an innovative biochemical preparation, researchers of EUROIMMUN AG have developed a new test system that exceeds the diagnostic quality criteria of all conventional Anti-dsDNA ELISA by far (Anti-dsDNA-NcX ELISA, EUROIMMUN AG, Luebeck, Germany). The secret of the innovation lies in the use of highly purified nucleosomes as the new linking substance. Since nucleosomes have a strong adhesive ability, even the smallest concentration of these is highly suited to couple isolated dsDNA to the surface of a microplate well. Poly-L-lysine and protamine sulphate have fallen into disuse, many face of a microplate well. Poly-L-lysine and protamine sulphate have fallen into disuse, many

In a clinical comparison study using 378 samples from patients with rheumatic diseases (209 of these with SLE) the Anti-dsDNA-NcX ELISA clearly demonstrated its superiority over the Anti-dsDNA RIA (Farr assay), showing an 8% higher sensitivity.

<table>
<thead>
<tr>
<th>Panel (Source: Charité Universitätsmedizin Berlin)</th>
<th>n</th>
<th>Anti-dsDNS NcX-ELISA positive</th>
<th>Anti-dsDNS RIA positive</th>
<th>Anti-dsDNS ELISA positive</th>
<th>IFT (Crithidia luciliae) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>209</td>
<td>125</td>
<td>108</td>
<td>88</td>
<td>57</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>209</td>
<td>59.8%</td>
<td>42.1%</td>
<td>27.4%</td>
<td></td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>88</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Progressive systemic sclerosis</td>
<td>81</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>169</td>
<td>98.2%</td>
<td>97.6%</td>
<td>95.9%</td>
<td></td>
</tr>
<tr>
<td>Sensitivity at 98 % specificity (according to ROC analysis)</td>
<td>378</td>
<td>65.8%</td>
<td>35.4%</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* Only 208 SLE sera were incubated on Crithidia luciliae

The Anti-dsDNA-NcX ELISA can be performed manually or fully automatically (EUROIMMUN Analyzer I). Other competent test systems such as the Farr assay and the indirect immunofluorescence test (substrate Crithidia luciliae) continue to be of importance in the clarification of discrepant serological and clinical results.
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Test characteristics Anti-dsDNA-NcX ELISA (IgG)

Linearity: The linearity of the Anti-dsDNA-NcX ELISA (IgG) was determined by assaying 8 serial dilutions of 4 serum samples. The linear regression was calculated, R² amounting to >0.95 in all samples. The Anti-dsDNA-NcX ELISA (IgG) is linear in at least the tested concentration range of 40 IU/ml to 757 IU/ml.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Correlation of the ELISA with conventional test systems: 209 SLE sera were investigated with the Anti-dsDNA-NcX ELISA, an Anti-dsDNA RIA and a conventional Anti-dsDNA ELISA. The clinical sensitivity of the Anti-dsDNA-NcX ELISA amounted to 60% and clearly exceeded the sensitivity determined for the Anti-dsDNA RIA (52%) and the Anti-dsDNA ELISA (42%).

Reference range: Levels of anti-dsDNA antibodies were investigated in 400 sera from healthy blood donors between 18 and 69 years of age (176 women, 224 men) using the EUROIMMUN ELISA. No differences with respect to age or gender were observed. The mean concentration of antibodies against dsDNA was 6.8 IU/ml (± 8.2 IU/ml of standard deviation) and the values ranged from 0.6 to 71.8 IU/ml. With a cut-off of 100 IU/ml none of the blood donors were anti-dsDNA positive.

ROC analysis: In an analysis of 209 SLE samples and 760 control sera (controls for specificity calculation plus 165 samples from RA patients, 26 samples from myositis patients and 400 samples from healthy blood donors) the following results were achieved:

Technical data:

Antigen Nucleosome-complexed (NcX) dsDNA coupled to the solid phase.

Calibration Quantitative, in international units per milliliter (IU/ml).

Sample dilution Serum or plasma; 1:201 in sample buffer.

Reagents Ready for use. Exception: wash buffer (10x). Colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits.

Test procedure 30 min / 30 min / 15 min. Room temperature. Fully automatable.

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

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

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