Anti-Gliadin (GAF-3X) ELISA

**Indication:** Test system for the in vitro determination of antibodies against gliadin-analogous fusion peptides in human serum or plasma for the diagnosis of the following diseases: gluten-sensitive enteropathy (coeliac disease, sprue), dermatitis herpetiformis (Duhring’s disease).

**Clinical significance:** Coeliac disease (or coeliac sprue) is an autoimmune disease caused in predisposed individuals by consumption of gluten-containing cereal products. The disease is characterised by atrophy of the small-intestinal villi, chronic diarrhoea and the consequences of malabsorption. Coeliac disease is associated with dermatitis herpetiformis (a skin disease characterised by subepidermal blisters) and complications during pregnancy. Known long-term damages are mainly osteoporosis and lymphoma of the small intestine.

**Application of the Anti-Gliadin (GAF-3X) ELISA:** Diagnosis of coeliac disease is based on the determination of antibodies against tissue transglutaminase (tTG; endomysium) and gliadin. With almost 100% sensitivity and specificity, IgA class antibodies against tTG have a very high diagnostic relevance. Antibodies against gliadin, however, have so far only been of limited use in the diagnosis of coeliac disease because they are also frequently found in healthy individuals. The antigen used in conventional test systems, which is based on native gliadin, is bound by unspecific antibodies (mainly of class IgG), which also occur in non-coeliac patients and healthy persons.

In 2004 Schwertz et al. identified different gliadin nonapeptides for the optimised diagnosis of coeliac disease (Clinical Chemistry 50(12): 2370-2375, 2004). Antibodies against these peptides show a very high specificity for coeliac disease and rarely occur in healthy individuals. Based on these findings, the EUROIMMUN Institute for Experimental Immunology developed a gliadin-analogous fusion peptide (GAF; consisting of 3 repetitive sequences) for use in the new Anti-Gliadin (GAF-3X) ELISA. For the evaluation of the new test system, a multi-centre study was performed, which produced the following results:

<table>
<thead>
<tr>
<th>Panel</th>
<th>n</th>
<th>Gliadin (GAF-3X) (IgA) positive</th>
<th>Gliadin (GAF-3X) (IgG) positive</th>
<th>Gliadin (IgA) positive</th>
<th>Gliadin (IgG) positive</th>
<th>tTG (IgA) positive</th>
<th>tTG (IgG) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coeliac disease (children &gt;1.25 years)</td>
<td>139</td>
<td>115</td>
<td>118</td>
<td>96</td>
<td>127</td>
<td>135</td>
<td>45</td>
</tr>
<tr>
<td>Duhring’s dermatitis herpetiformis (adults, diet unknown)</td>
<td>13</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>139</td>
<td>82.7%</td>
<td>84.9%</td>
<td>65.1%</td>
<td>91.4%</td>
<td>92.1%</td>
<td>32.4%</td>
</tr>
<tr>
<td>Gastroenteropathies, negative biopsy for coeliac disease</td>
<td>129</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>36</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Chronic-inflammatory bowel disease, negative biopsy for coeliac disease</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Specificity</td>
<td>145</td>
<td>95.2%</td>
<td>97.3%</td>
<td>93.8%</td>
<td>71.5%</td>
<td>93.1%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Sensitivity at 95% specificity</td>
<td>284</td>
<td>84.9%</td>
<td>94.2%</td>
<td>61.8%</td>
<td>33.1%</td>
<td>36.4%</td>
<td>64.0%</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>200</td>
<td>6</td>
<td>1</td>
<td>31</td>
<td>19</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>200</td>
<td>9</td>
<td>4</td>
<td>29</td>
<td>11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Systemic lupus erythematous</td>
<td>100</td>
<td>3</td>
<td>7</td>
<td>23</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Progressive systemic sclerosis</td>
<td>126</td>
<td>6</td>
<td>3</td>
<td>18</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Specificity</td>
<td>771</td>
<td>96.0%</td>
<td>97.3%</td>
<td>95.7%</td>
<td>87.3%</td>
<td>97.7%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

1 Prof. Mothes, University clinic of Leipzig  
2 Prof. Hiepe, University clinic Charité, Berlin  
3 Prof. Zimmer, University clinic of Greisa-Marburg  
4 Dr. Uhlig, University clinic of Leipzig  
5 Prof. Richter, Childrens’ clinic of the Municipal clinic St. George Leipzig  
6 Prof. Hauser, University clinic of Graz  
7 Prof. Stern, University clinic of Tubingen  
8 Dr. Laass, University clinic of Dresden

The results of the study clearly show that the sensitivity (at a specificity of 95%) and specificity of the new Anti-Gliadin (GAF-3X) ELISA are significantly higher than those achieved with the conventional Anti-Gliadin ELISA. Gliadin (GAF-3X) antibodies of class IgG are even more sensitive and specific than gliadin (GAF-3X) antibodies of class IgA and have a specificity which is comparable to tTG antibodies of class IgA.

For optimal diagnostic results, antibodies against tTG (IgA) and gliadin (GAF-3X) should be investigated in parallel. This procedure gives the highest serological hit rate. Moreover, the Anti-Gliadin (GAF-3X) ELISA (IgG) is suited for the identification of patients with IgA deficiency. The determination of these antibodies can be used for the control of the disease activity during its course and for the monitoring of a gluten-free diet or a gluten loading test.
Test Characteristics

Anti-Gliadin (GAF-3X) ELISA

Linearity: The linearity of the Anti-Gliadin (GAF-3X) ELISA was determined by assaying 4 serial dilutions of 5 serum samples. The linear regression was calculated, $R^2$ amounting to >0.95 in all samples. The Anti-Gliadin (GAF-3X) ELISA is linear at least in the tested concentration range (IgA: 9 RU/ml to 195 RU/ml; IgG: 10 RU/ml to 183 RU/ml).

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) 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. The mean value of the intra-assay CVs was 5.0% for IgA (range: 3.6% to 7.0%) and 5.3% for IgG (range: 4.4% to 6.4%). The mean value of the inter-assay CVs was 6.0% for IgA (range: 4.4% to 8.9%) and 6.4% for IgG (range: 4.1% to 11.4%).

Correlation with the conventional Anti-Gliadin ELISA (IgA, IgG): In a comparison study 139 sera from coeliac patients and 145 control sera (129 sera from patients with gastrenteropathies, negative biopsy for coeliac disease; 16 sera from patients with chronic-inflammatory bowel diseases, negative biopsy for coeliac disease) were investigated with the Anti-Gliadin (GAF-3X) ELISA (IgA, IgG) and the Anti-Gliadin ELISA (IgA, IgG). The following data were achieved:

Reference range: Levels of anti-gliadin (GAF-3X) antibodies were investigated in 400 sera from healthy blood donors between 18 and 68 years of age (176 women, 224 men) using the EUROIMMUN ELISA. At a cut-off of 25 RU/ml, 97.7% (IgA) and 98.0% (IgG) of blood donors were negative for anti-gliadin (GAF-3X).

ROC analysis: The following data was achieved in the analysis of 139 samples from coeliac patients and 1321 control samples (control samples used for specificity calculation see table page 1, and in addition 100 samples of SLE patients, 50 samples of RA patients and 400 samples of blood donors):

Technical data:

Antigen Trimer of a deamidated gliadin-analogous fusion peptide
Calibration Quantitative, in relative units per milliliter (RU/ml).
  - Calibration serum 1: 200 RU/ml
  - Calibration serum 2: 25 RU/ml; cut-off
  - Calibration serum 3: 2 RU/ml
Sample dilution Serum or plasma; 1:201 in sample buffer.
Reagents Ready for use, with exception of the 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 reagent wells. Kit includes all necessary reagents.
Order no. EV 3011-9601 A (IgA), EV 3011-9601 G (IgG)