ENZYME IMMUNOASSAYS FOR THE DIAGNOSIS OF COELIAC DISEASE, FOOD INTOLERANCES AND INFLAMMATORY BOWEL DISEASE

Transglutaminase
Deamidated gliadin
Gliadin
Milk
ASCA

ELISA kits are optimized and validated for detection of IgA, IgG and IgM antibodies in human serum and plasma
Coeliac sprue (Coeliac disease) is a common term for a cosmopolitan disease that occurs in people of all ages. It is a hereditary autoimmune disease caused by gluten intolerance. The main symptoms include inflammatory changes in the small intestine mucosa, diarrhea, anaemia, weight loss and general disorders in somatic and psychic development. If gluten is not completely and permanently removed from patient’s food, their immune system gets exhausted, the disease affects other organs and further autoimmune diseases and complications may develop, most of them being life threatening.

Gluten intolerance (simple) should not be confused with coeliac disease. Gluten intolerance can proceed in parallel with cow’s milk intolerance and intestinal mucosa changes, without activation of transglutaminase. In this case, there is no progress of coeliac disease.

Cow’s milk intolerance is a disease affecting children and adults. It is caused by intolerance to cow’s milk proteins (β-lactoglobulin, α-lactalbumin, casein). General symptoms include vomiting, diarrhea and abdomen aches, and malabsorption syndrome, respectively.

ASCA (antibodies to Saccharomyces cerevisiae), which react against mannan antigen in the cell wall of the yeast, are very specific for Crohn’s disease. Crohn’s disease together with ulcerative colitis belongs to a group of nonspecific inflammatory bowel diseases. Crohn’s disease is a chronic disease of the whole digestive tract, which can cause extraintestinal complications. Inflammation of the gastrointestinal tract leads to absorption problems developing diarrhea and malabsorption syndrome.
Coeliac Sprue
The diagnostics of the disease is based on clinical manifestation, enterobiopsy and laboratory tests. Detection of highly specific IgA and IgG antibodies to Deamidated gliadin and Transglutaminase is very important and useful for proper diagnosis of coeliac disease and also for monitoring of the effects of gluten-free diet treatment.

Intolerance to Glutein (simple)
Detection of specific IgA and IgG antibodies to gluten (or rather, to its specific α-gliadin fraction), is a key finding, leading to a proper diagnosis of gliadin intolerance and also to monitoring of gluten-free diet treatment effects.

Cow’s Milk Intolerance
Detection of specific IgA, IgG and IgM antibodies to cow’s milk proteins is an essential finding in differential diagnostics of gastro-enteric diseases, in children especially.

ASCA
Diagnosis of the disease is based on clinical manifestation, results of laboratory tests, endoscopic and imaging methods. Detection of highly specific IgA and IgG antibodies to Saccharomyces cerevisiae (ASCA) together with detection of anti-neutrophil cytoplasmic antibodies (ANCA) is valuable in a differential diagnosis of inflammatory bowel disease (IBD). ASCA are detected in 60-80% of Crohn’s patients and in 5–15% of those with ulcerative colitis. ASCA can be increased also in patients with celiac disease etc.

Test Principle
The assays are based on a sandwich type of ELISA method.

![Sandwich ELISA Diagram](Image)
**Antigens**

Transglutaminase – human tissue recombinant tTG
Deamidated gliadin – deamidated gliadin peptide DGPx1
Gliadin – antigenic extract of gluten, with specific protein antigens, especially α-gliadin
Milk – whole delipidated antigen, prepared from cow’s milk, rich in proteins (casein, α-lactalbumin and β-lactoglobulin)
ASCA - high purified mannan from *Saccharomyces cerevisiae*

**User Comfort**

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF and calibrators included
- Semiquantitative evaluation of results (Index of Positivity) or quantitative evaluation of results (U/ml)
- Easy assay procedure

**EIA Gliadin DA IgA**

![Graph showing the relationship between U/ml and O.D. values.](image)
**Summary of EIA Protocol**

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Test steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dilute samples serum/plasma (1+100); for ASCA (1+50)</td>
</tr>
<tr>
<td>2</td>
<td>Pipette Controls and diluted samples 100 µl Blank = 100 µl Sample diluent; for ASCA Blank = empty well</td>
</tr>
<tr>
<td>3</td>
<td>Incubate 30 minutes at 37°C</td>
</tr>
<tr>
<td>4</td>
<td>Aspirate and wash the wells 4 times</td>
</tr>
<tr>
<td>5</td>
<td>Add 100 µl Conjugate Including blank; for ASCA Blank = empty well</td>
</tr>
<tr>
<td>6</td>
<td>Incubate 30 minutes at 37°C for ASCA 15 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Aspirate and wash the wells 4 times</td>
</tr>
<tr>
<td>8</td>
<td>Add 100 µl Substrate (TMB-Complete) Including blank</td>
</tr>
<tr>
<td>9</td>
<td>Incubate 15 minutes at 37°C</td>
</tr>
<tr>
<td>10</td>
<td>Add 100 µl Stopping solution Including blank</td>
</tr>
<tr>
<td>11</td>
<td>Read colour intensity at 450 nm</td>
</tr>
</tbody>
</table>

**Test Characteristics**

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Diagnostic Sensitivity</th>
<th>Diagnostic Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA Transglutaminase IgA</td>
<td>97.7%</td>
<td>97.7%</td>
</tr>
<tr>
<td>EIA Transglutaminase IgG</td>
<td>96.2%</td>
<td>97.7%</td>
</tr>
<tr>
<td>EIA Gliadin DA IgA</td>
<td>95.5%</td>
<td>97.7%</td>
</tr>
<tr>
<td>EIA Gliadin DA IgG</td>
<td>97.7%</td>
<td>97.7%</td>
</tr>
<tr>
<td>EIA Gliadin IgA</td>
<td>95.5%</td>
<td>95.5%</td>
</tr>
<tr>
<td>EIA Gliadin IgG</td>
<td>95.5%</td>
<td>95.5%</td>
</tr>
<tr>
<td>EIA Milk IgA</td>
<td>95.2%</td>
<td>95.5%</td>
</tr>
<tr>
<td>EIA Milk IgG</td>
<td>95.0%</td>
<td>95.2%</td>
</tr>
<tr>
<td>EIA Milk IgM</td>
<td>95.2%</td>
<td>95.5%</td>
</tr>
<tr>
<td>EIA ASCA IgA</td>
<td>98.5%</td>
<td>98.1%</td>
</tr>
<tr>
<td>EIA ASCA IgG</td>
<td>98.6%</td>
<td>99.1%</td>
</tr>
</tbody>
</table>

**Results Interpretation**

**Coeliac Sprue – Diagnostic Scheme**

- **Low risk Screening Asymptomatic population**
  - Serology (gliadin, gliadin deamidated, tissue transglutaminase, endomysium antibodies)
    - Serology NEG
    - Serology POS
- **High risk Susp. coeliac sprue, Family coeliac sprue, Malabsorption syndrom**
  - Serology (gliadin, gliadin deamidated, tissue transglutaminase, endomysium antibodies)
    - Serology NEG
    - Serology POS
    - Enterobiopsy
  - Enterobiopsy
    - Serology NEG
    - Serology POS
      - Serology NEG
      - Serology POS
      - Repeat enterobiopsy
- **None Coeliac sprue**
  - Other causes of Mucose membrane atrophy
    - Coeliac sprue confirmed
    - Histology POS
  - Other causes eliminated
    - Coeliac sprue confirmed
    - Histology POS
- **Another disease proven**
  - Coeliac sprue confirmed
  - Histology POS
### Advantages

- Identical assay procedure
- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Short total assay time
- Quantitative evaluation available
- Ready for automation
- Customer support

### Clinical Data

#### Coeliac Sprue – Correlation of Methods

**EIA Transglutaminase IgA and IFA EmA IgA antibodies correlation**

<table>
<thead>
<tr>
<th>EmA IgA</th>
<th>EIA Transglutaminase IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>23</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EmA IgA</th>
<th>EIA Transglutaminase IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>53</td>
</tr>
</tbody>
</table>

**Number of corresponding results: 96.2%**

**EIA Transglutaminase IgA and EIA Gliadin DA IgA antibodies correlation**

<table>
<thead>
<tr>
<th>Gliadin DA IgA</th>
<th>EIA Transglutaminase IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>25</td>
</tr>
<tr>
<td>+</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gliadin DA IgA</th>
<th>EIA Transglutaminase IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>+</td>
<td>50</td>
</tr>
</tbody>
</table>

**Number of corresponding results: 93.75%**

**EIA Gliadin DA IgA and IFA EmA IgA antibodies correlation**

<table>
<thead>
<tr>
<th>EmA IgA</th>
<th>EIA Gliadin DA IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>42</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EmA IgA</th>
<th>EIA Gliadin DA IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>44</td>
</tr>
</tbody>
</table>

**Number of corresponding results: 98.85%**

The borderline results were excluded from evaluation.
EIA ASCA IgA and IgG

Comparison of the detection of anti-Saccharomyces cerevisiae antibodies in patients with confirmed Crohn's disease (n = 23) and ulcerative colitis (n = 22) using TestLine EIA kits.

- Crohn's disease: 82.6%
- Ulcerative colitis: 13.6%
### ELISA – Food Intolerance

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIA096</td>
<td>EIA Gliadin IgA</td>
<td>96</td>
</tr>
<tr>
<td>GIG096</td>
<td>EIA Gliadin IgG</td>
<td>96</td>
</tr>
<tr>
<td>MiA096</td>
<td>EIA Milk IgA</td>
<td>96</td>
</tr>
<tr>
<td>MiG096</td>
<td>EIA Milk IgG</td>
<td>96</td>
</tr>
<tr>
<td>MiM096</td>
<td>EIA Milk IgM</td>
<td>96</td>
</tr>
</tbody>
</table>

### ELISA – Coeliac Disease

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
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<tbody>
<tr>
<td>GDA096</td>
<td>EIA Gliadin DA IgA</td>
<td>96</td>
</tr>
<tr>
<td>GDG096</td>
<td>EIA Gliadin DA IgG</td>
<td>96</td>
</tr>
<tr>
<td>tTA096</td>
<td>EIA Transglutaminase IgA</td>
<td>96</td>
</tr>
<tr>
<td>tTG096</td>
<td>EIA Transglutaminase IgG</td>
<td>96</td>
</tr>
</tbody>
</table>

### ELISA – IBD

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>ScA096</td>
<td>EIA ASCA IgA</td>
<td>96</td>
</tr>
<tr>
<td>ScG096</td>
<td>EIA ASCA IgG</td>
<td>96</td>
</tr>
</tbody>
</table>

### Ordering Information

#### ELISA – Food Intolerance

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</tr>
<tr>
<td>GIG096</td>
<td>EIA Gliadin IgG</td>
<td>96</td>
</tr>
<tr>
<td>MiA096</td>
<td>EIA Milk IgA</td>
<td>96</td>
</tr>
<tr>
<td>MiG096</td>
<td>EIA Milk IgG</td>
<td>96</td>
</tr>
<tr>
<td>MiM096</td>
<td>EIA Milk IgM</td>
<td>96</td>
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### ELISA – Coeliac Disease

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### Contact

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.