Indication: Test system for the in vitro determination of antibodies against endomysium (EmA) and gliadin-analogue fusion peptides (GAF) 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 damage includes mainly osteoporosis and lymphoma of the small intestine.

Application of the EUROPLUS™ Liver (primate) / Gliadin (GAF-3X): For the diagnosis of coeliac disease, antibodies against endomysium, tissue transglutaminase and gliadin can be determined. With almost 100% sensitivity and specificity, IgA class antibodies against endomysium have a very high diagnostic relevance. However, in the past, antibodies against gliadin were only of limited use in the diagnosis of coeliac disease because they were also frequently found in healthy individuals. These unspecific reactions were caused by purified, native gliadin used in conventional test systems.

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 showed a very high specificity for coeliac disease and rarely occurred in healthy individuals. Based on these findings, the EUROIMMUN Institute for Experimental Immunology developed a gliadin-analogue fusion peptide (GAF-3X) for use in the new EUROPLUS™ Anti-Gliadin (GAF-3X) IIFT. Antibodies against gliadin (GAF-3X) have a very high sensitivity and specificity, particularly for IgG. The new EUROPLUS™ Anti-Gliadin (GAF-3X) IIFT therefore also enables identification of coeliac disease patients who have a selective IgA-deficiency. Around 5% of patients do not exhibit IgA antibodies. A reliable IgG test is therefore very important.

Antibody concentrations were measured in 234 (IgA) and 226 (IgG) sera using the EUROPLUS™ Anti-Gliadin (GAF-3X) IIFT and the Anti-Gliadin (GAF-3X) ELISA. The qualitative results of the test systems were 94.9% (IgA) and 99.1% (IgG) in agreement.

The EUROPLUS™ Liver (primate)/Gliadin (GAF-3X) enables determination of two different coeliac-specific antibodies in one test: EmA and anti-gliadin (GAF-3X): By combining classical tissue sections (e.g. primate liver, oesophagus, intestine) with defined gliadin (GAF-3X) micro-droplets a higher serological hit rate can be achieved than by using one substrate alone.
Test characteristics
EUROPLUS® Liver (primate) / Gliadin (GAF-3X)

Test principle: The indirect immunofluorescence test is an in vitro assay for the determination of specific antibodies against endomysium (EmA) and gliadin (GAF-3X). The coated BIOCHIPs are incubated with diluted patient samples. In the case of positive reactions, specific antibodies of the class IgA and IgG will bind to the antigen. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

Test procedure: EUROIMMUN BIOCHIP slides are incubated using the proprietary TITERPLANE™ Technique. Results are evaluated by fluorescence microscopy. Incubation of the substrates with the positive and negative controls provided in each kit verifies correct performance of the test and aids evaluation.

Specificity and sensitivity:

<table>
<thead>
<tr>
<th>Substrate / Ig class</th>
<th>Reference</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (primate): Anti-Endomysium (IgA)</td>
<td>IIFT: Oesophagus (primate) (n=68, Germany)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Liver (primate): Anti-Endomysium (IgG)</td>
<td>IIFT: Oesophagus (primate) (n=68, Germany)</td>
<td>96%</td>
<td>91%</td>
</tr>
<tr>
<td>Gliadin (GAF-3X) (IgA)</td>
<td>Healthy blood donors (n=200, Germany) Anti-Gliadin (GAF-3X) ELISA (n=122 samples precharacterised as positive, Germany)</td>
<td>99%</td>
<td>95%</td>
</tr>
<tr>
<td>Gliadin (GAF-3X) (IgG)</td>
<td>Healthy blood donors (n=200, Germany) Anti-Gliadin (GAF-3X) ELISA (n=114 samples precharacterised as positive, Germany)</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Reference range: titer 1:<10

Inter-lot reproducibility: Inter-lot reproducibility was tested with more than 10 different lots. The deviation in the fluorescence intensity of the IIFT amounted to no more than ±1 intensity level for all samples.

Technical data:

Antigen substrate: Frozen sections of primate liver; trimer of a deamidated gliadin-analogue fusion peptide (GAF-3X).

Sample dilution: Serum or plasma.
Qualitative evaluation: 1:10
Quantitative evaluation: 1:10/100/1000 etc.

Test procedure: 30min (sample) / 30min (conjugate), room temperature.

Microscopy: Objektive: 20 x, excitation filter: 488 nm, colour separator: 510 nm, blocking filter: 520 nm, light source: EUROIMMUN LED or mercury vapour lamp, 100 W

Test kit format: 10 or 20 slides, each containing 3, 5 or 10 test fields. The kits include all necessary reagents.

Reagents: Ready for use, with the exception of the PBS Tween (for dilution and washing step).

Order number: FA 1914-1005-1 A or G (example for test kit containing 10 slides each with 5 test fields)

Related products:
FA 1911-1: EUROPLUS® Oesophagus/Gliadin (GAF-3X)
FA 1913-1: EUROPLUS® Intestine/Gliadin (GAF-3X)
FA 1913-2: EUROPLUS® Oesophagus/Intestine/Gliadin (GAF-3X)
FA 1913-6: EUROPLUS® Intestine/Oesophagus/Liver/Gliadin (GAF-3X)
FA 1914-3: EUROPLUS® Intestine/Liver/Gliadin (GAF-3X)