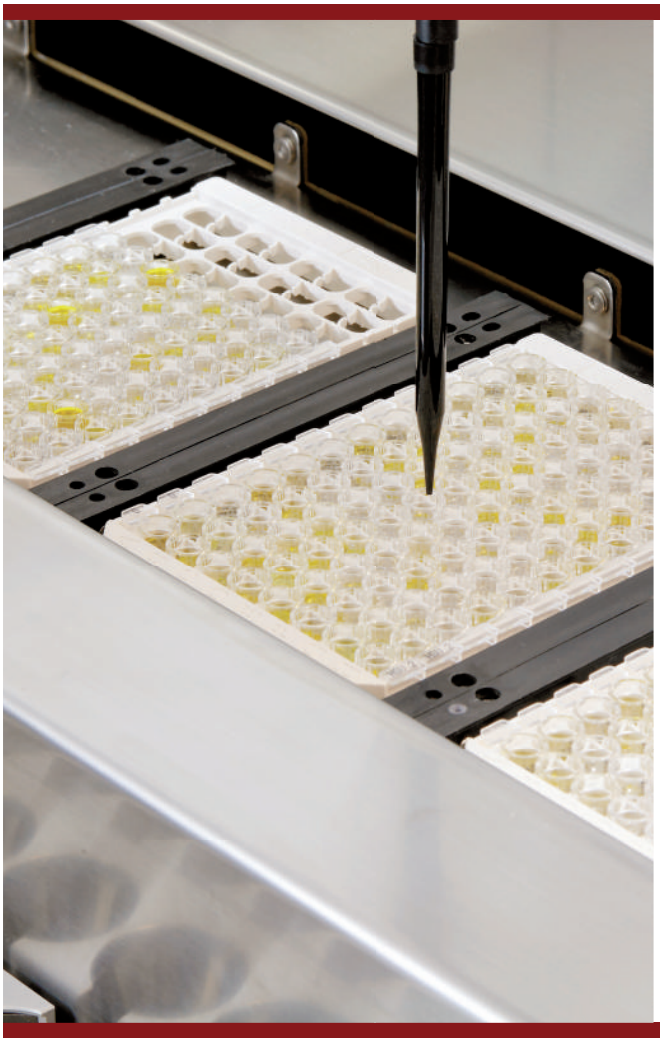
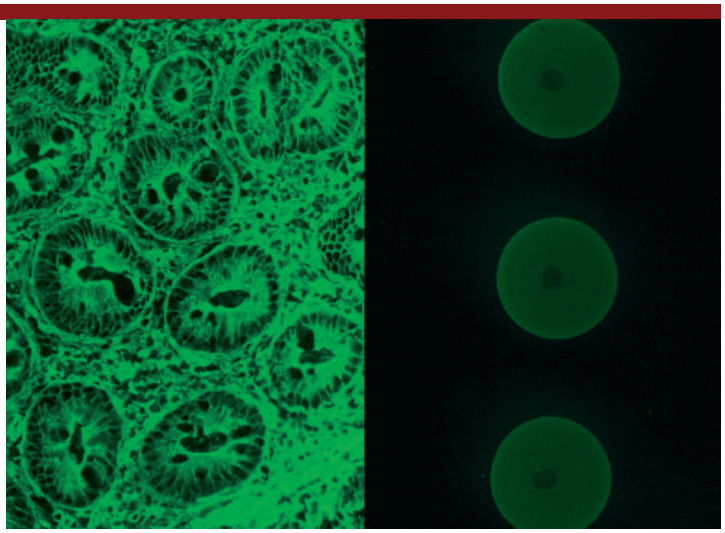
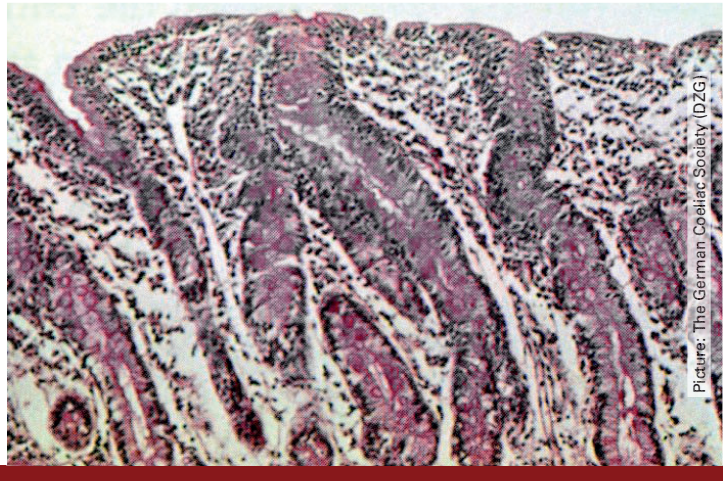
EUROIMMUN Medizinische Labordiagnostika AG

Diagnosing coeliac disease
All test systems from one manufacturer

Comprehensive range of tests for diagnostics, therapy monitoring and risk assessment







Picture: The German Coeliac Society (DZG)

- **Anti-Tissue Transglutaminase + Anti-Gliadin (GAF-3X) ELISA**
- **Anti-Endomysium IIFT + Anti-Gliadin (GAF-3X) EUROPLUS**
- **Anti-Tissue Transglutaminase + Anti-Gliadin (GAF-3X) EUROLINE**
- **HLA-DQ2 / DQ8 EUROArray**

Definition and classification of coeliac disease

Coeliac disease is a systemic autoimmune disease with pronounced genetic predisposition which may affect different organ systems. The prevalence of the disease is estimated to amount to approximately 1%, whereby experts suppose there are a large number of unreported cases which are not diagnosed due to atypical or mild symptoms. Coeliac disease is triggered by consumption of gluten, which accounts for around 90% of the protein contents of many grain seeds. The disease manifests mostly by severe inflammation and damage of the mucous membrane of the small intestine (enteropathy). In conjunction with the resulting disturbance in the nutrient absorption, a broad spectrum of clinical gastrointestinal and non-gastrointestinal symptoms can develop (among others chronic diarrhoea, vomiting, abdominal pain, cramps, short stature, weight loss, delayed puberty, spontaneous abortions, anaemia and osteoporosis). Moreover, the clinical manifestation of coeliac disease may include a chronic rash in the form of dermatitis herpetiformis (Duhring's disease).

Classification of coeliac disease *	Malabsorption	Unspec. symptoms	Enteropathy	Spec. antibodies	Genetic predisposition
Symptomatic	+	+	+	+	+
Classic	+	+/–	+	+	+
Subclinical	–	–	+	+	+
Refractory (only adults)	–	+/–	+	+	+
Potential	–	–	–	+	+

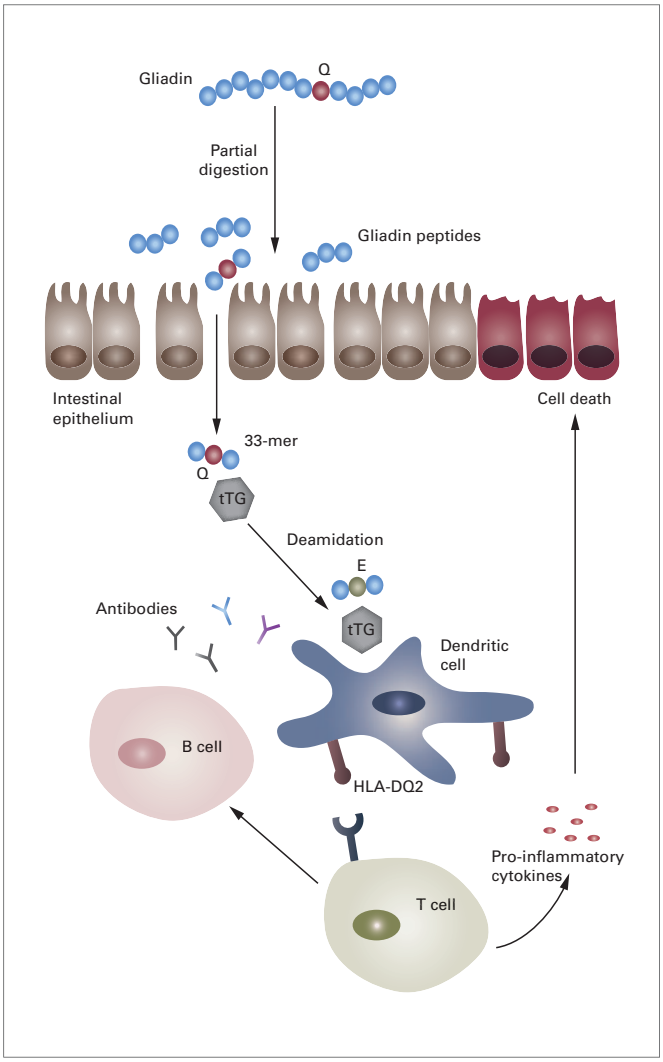
* OSLO classification Felber et al., Results from a S2k consensus conference (...). Z Gastroenterol 2014; 52: 711-743, based on Ludvigsson et al., Gut 2013, 62: 43-52

Pathogenesis of coeliac disease

Genetic as well as environmental factors contribute to the development of coeliac disease. Enteropathy, which is characteristic of coeliac disease, is caused by an overreaction of the immune system to gluten components, especially the so-called gliadin.

Gliadin is only partially digested in the small intestine. If the intestinal epithelium presents gaps, as is typical in patients with coeliac disease, the resulting gliadin fragments (peptides, consisting of 33 amino acids, 33-mer) can pass the intestinal barrier and reach the underlying connective tissue. There, the enzyme tissue transglutaminase (tTG) modifies (deamidates) the amino acid glutamine (Q) into the amino acid glutamate (E) at specific sites of the gliadin peptides. With the modification, the peptides acquire their immunological effect if the genetic predisposition is present. Especially two genetic variants (DQ2 and DQ8) of the human leukocyte antigen system (HLA system) are associated with the immune reaction. Dendritic cells phagocyte the complex of tissue transglutaminase and deamidated gliadin peptides and, if they express HLA-DQ2 or HLA-DQ8 on their surface, can present it together with the HLA molecules to the T cells of the immune system. The T cells activate the B cells, which then produce antibodies against the deamidated gliadin peptides (so-called coeliac disease-specific anti-gliadin fragment antibodies CD-AGFA) and against the body-own tissue transglutaminase. In addition, the T cells secrete pro-inflammatory cytokines which cause an inflammatory reaction in the tissue.

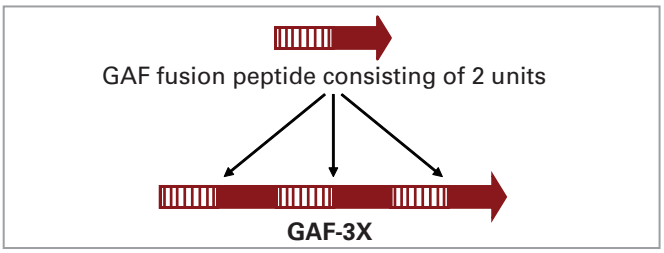
The immunological overreaction and the inflammation of the epithelium of the small intestine lead to apoptosis of the enterocytes, atrophy of the villi, and broadening of the intestinal crypts (hyperplasia). The intestinal mucosa, damaged in such way, is no longer able to absorb sufficient nutrients from the digested food and carry them over into the blood stream.



Overview of parameters and methods for coeliac disease diagnostics

Serological determination of coeliac disease-specific antibodies

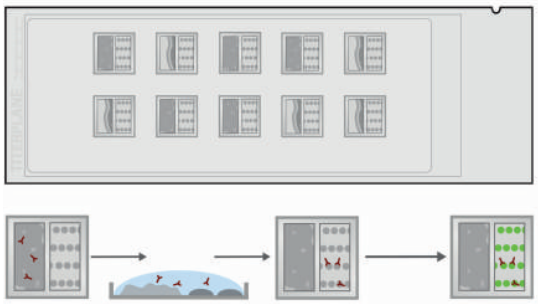
Antibodies against endomysium (EmA) are considered as very specific and sensitive markers for the diagnosis of coeliac disease. They can be detected by IIFT on tissue sections of liver, oesophagus, or intestine (primate). The target antigen of EmA is tissue transglutaminase (tTG). Anti-tTG antibodies can be determined using antigen-coated ELISA microplate strips or EUROLINE immunoblots. The antibodies associated with coeliac disease also include those against deamidated epitopes of gliadin peptides (anti-DGP antibodies; CD-AGFA). These can also be detected by ELISA or EUROLINE as well as by means of the monospecific EUROPLUS substrate. Anti-tTG antibodies and EmA of immunoglobulin class A (IgA) are particularly relevant for diagnostics. If a general IgA deficiency is present – a condition which is observed especially often (above average) in patients with coeliac disease – CD-AGFA of immunoglobulin class G (IgG) are considered an important alternative indicator of coeliac disease. Generally, the diagnostic determination of coeliac disease-specific antibodies must be performed under normal, gluten-containing diet since the antibodies disappear with a gluten-free diet.



In order to provide specific and sensitive detection of coeliac disease-associated gliadin fragment antibodies, EUROIMMUN has developed the antigen substrate gliadin (GAF-3X). This consists of three deamidated gliadin-analogue fusion peptides (GAF) in a row. GAF consists of two synthetic nonapeptides which have proven particularly specific and sensitive for the detection of coeliac disease-specific antibodies amongst the 51 peptides tested (Schwertz et al., Clin Chem 2004; 50(12): 2370-2735). Owing to the reduction of the substrate to two short gliadin peptides, unspecific reactions are prevented and the specificity of the test system is increased. Since GAF is used as a trimer instead of a monomer, also the sensitivity of the test for the detection of the relevant antibodies is optimised.

Co-incubation of biopsy samples and monospecific antigen substrates

Disease-specific antibodies are not only detectable in serum, but also in the inflamed intestinal tissue of patients with coeliac disease. This is especially helpful for pathologists who only have at their disposal biopsies and no serum samples from the patient. In order to determine tissue-bound antibodies, a new method was developed, the co-incubation: A frozen section of a patient biopsy (donor substrate) is incubated alongside an antigen (acceptor substrate) on a reaction field of the slide. During the co-incubation, the antibodies contained in the biopsy are dissolved from the tissue. These diffuse to the adjacent acceptor substrate and bind to the antigen. The antibodies are then detected by a FITC-conjugated anti-human antibody. By performing the co-incubation, tissue-bound IgA antibodies against GAF-3X could be detection both in seropositive and in a portion of the seronegative patients with coeliac disease (publication in progress).

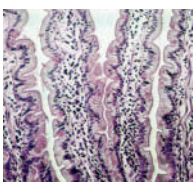


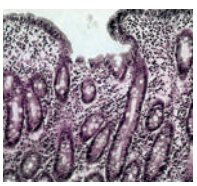
Histological investigation of a biopsy of the small intestine

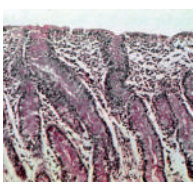
Tissue samples are usually taken from different sections of the duodenum, in an endoscopy. The lesions are assessed based on the Marsh criteria, according to the amount of intraepithelial leukocytes (IEL), and the state of the villi and crypts. They are, however, not specific for coeliac disease, but can also develop in other enteropathies.

Assessment of the histopathological severity according to Marsh.
Type 0: IEL, villi and intestinal crypts normal
Type 1: IEL increased, villi and intestinal crypts normal
Type 2*: IEL increased, intestinal crypts hyperplastic, villi normal
Type 3*: IEL increased, intestinal crypts hyperplastic, villi atrophic

**diagnostically relevant for coeliac disease*


Marsh type 0


Marsh type 2-3


Marsh type 3

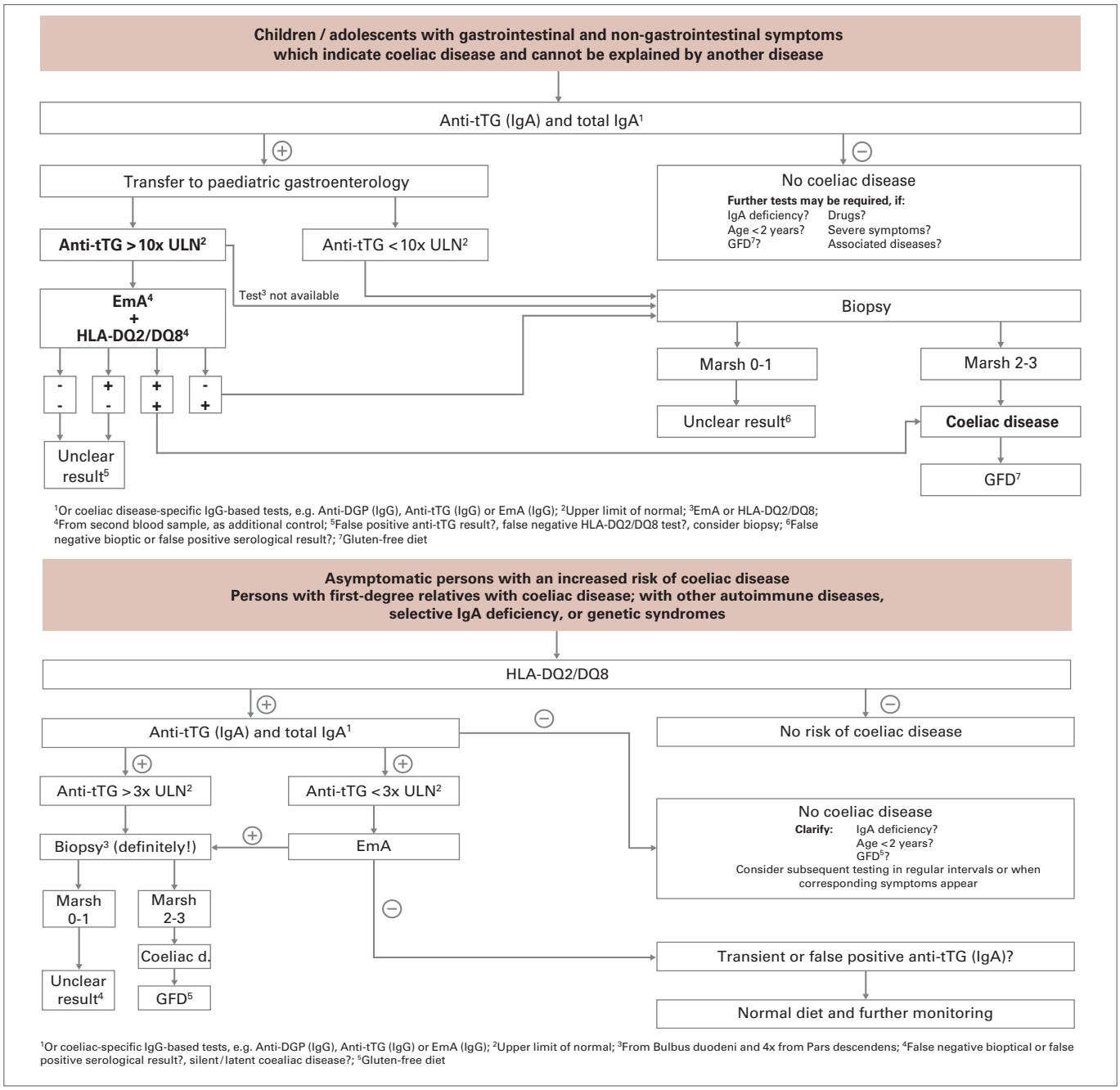
Photos: The German Coeliac Society (DZG)

Determination of HLA types

The determination of HLA types with molecular genetic test systems such as microarrays is especially relevant in symptomatic patients with unclear diagnosis or asymptomatic persons at risk. Coeliac disease can be excluded with very high probability when the HLA types DQ2 and DQ8 are not present in a patient.

ESPGHAN guidelines for the diagnosis of coeliac disease

In 2012, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition published new guidelines for the diagnosis of coeliac disease which enable a high diagnostic accuracy and reduce stress for the patients, e.g. owing to biopsies (Husby et al., JPGN 2012; 54: 136-160). If the titer of the anti-tTG IgA antibodies determined by ELISA exceeds 10 times the upper limit of normal (>10x ULN), a biopsy can be spared by performing further, non-invasive tests (e.g. EmA IIFT, HLA-DQ determination). A biopsy is then only required with unclear results. EUROIMMUN tests systems have proven suitable for this approach in several studies (see page 8). They showed the highest serological hit rate and help to make an optimal diagnosis.



EUROIMMUN test systems for the diagnosis of coeliac disease

I. ELISA test systems for coeliac disease

Anti-Tissue Transglutaminase ELISA

The serological determination of disease-associated antibodies of immunoglobulin class IgA against tTG is considered the most specific indicator for coeliac disease. Alongside qualitative antibody detection, the Anti-Tissue Transglutaminase ELISA also enables quantitative titer determination. In investigations of different panels the ELISA showed a very high sensitivity at a specificity of ≥98% for the determination of anti-tTG IgA antibodies.

Panel	n	Anti-Tissue Transglutaminase ELISA positive	
		IgA	IgG
Active coeliac disease (age: 0-18 years, bioptically tested)	183	179 (97.8 %)	58 (31.7 %)
Active coeliac disease (age: 1-54 years, bioptically tested)	58	58 (100.0 %)	9 (15.5 %)
Dermatitis herpetiformis	36	28 (77.8 %)	1 (2.8 %)
Sensitivity	**277**	**95.7 %**	**24.5 %**
Gastroenteropathies, bioptically negative for coeliac disease	243	12 (4.9 %)	2 (0.8 %)
Chronic inflammatory bowel diseases, bioptically negative for coeliac disease	55	2 (3.6 %)	0 (0.0 %)
Rheumatoid arthritis	300	1 (0.3 %)	0 (0.0 %)
Sjögren's syndrome	200	3 (1.5 %)	0 (0.0 %)
Systemic lupus erythematosus	150	0 (0.0 %)	0 (0.0 %)
Progressive systemic sclerosis	126	4 (3.2 %)	1 (0.8 %)
Bullous pemphigoid	30	1 (3.3 %)	0 (0.0 %)
Linear IgA dermatosis	22	0 (0.0 %)	0 (0.0 %)
Specificity	**1126**	**98.0 %**	**99.7 %**

Anti-Gliadin (GAF-3X) ELISA

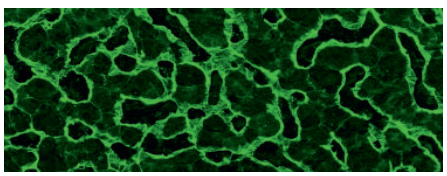
Conventional anti-n(ative) gliadin ELISAs are considered to be not very specific since antibodies against native gliadin are also found in healthy individuals. Antibodies against the gliadin fragments used in the Anti-Gliadin (GAF-3X) ELISA, however, are highly specific for coeliac disease patients. In a retrospective study by Prause et al., the EUROIMMUN test systems Anti-Gliadin (GAF-3X) ELISA, Anti-nGliadin ELISA and Anti-tTG ELISA were compared (J Pediatr Gastroenterol Nutr 2009; 49(1): 52-58). Sera of 142 children with active coeliac disease (serologically confirmed, Marsh 2 or 3) and 160 controls (bioptically confirmed, 19 patients with chronic inflammatory bowel diseases) were investigated for IgA and IgG antibodies. The Anti-Gliadin (GAF-3X) ELISA was generally superior to the Anti-nGliadin ELISA with respect to sensitivity and specificity for both IgA and IgG antibodies. Moreover, the Anti-Gliadin (GAF-3X) ELISA (IgG) was more sensitive and specific than the Anti-Gliadin (GAF-3X) ELISA (IgA) and the Anti-tTG ELISA (IgG) and provided results which were comparable to those of the Anti-tTG ELISA (IgA). A combination of the Anti-Gliadin (GAF-3X) ELISA (IgG) and the Anti-tTG ELISA (IgA) increased the accuracy of the results.

n = 302	ELISA	Sensitivity	Specificity
IgA	Anti-nGliadin (native)	73.9 %	91.9 %
	Anti-Gliadin (GAF-3X)	87.3 %	93.1 %
	Anti-tTG	95.1 %	98.1 %
IgG	Anti-nGliadin (native)	88.0 %	80.0 %
	Anti-Gliadin (GAF-3X)	95.1 %	94.4 %
	Anti-tTG	87.3 %	86.3 %

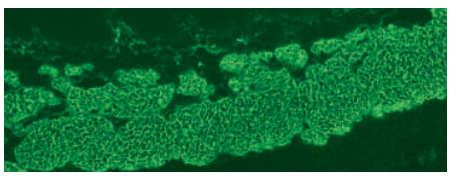
II. Indirect immunofluorescence tests (IIFT) for coeliac disease

Anti-Endomysium IIFT

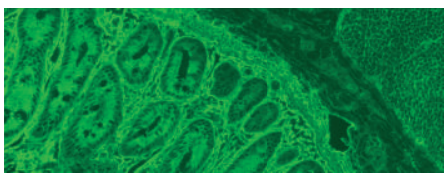
The Anti-Endomysium IIFT (IgA) is considered an especially specific test for the diagnosis of coeliac disease. The actual target antigen is tTG. Endomysium is a layer of connective tissue which surrounds the muscle cells of the skeletal musculature and the smooth muscle in hollow organs and blood vessels. Standard substrates for the detection of EmA in IIFT are tissue sections of primate oesophagus, small intestine or primate liver. The substrates are offered in the form of miniaturised BIOCHIPs which can be variably combined on one test field (BIOCHIP Mosaic).



Liver: Fluorescence of the vessel walls of the intralobular sinusoids.



Oesophagus: Broad fluorescent layer underneath the mucous membrane epithelial, honeycomb-like fluorescence in the lamina muscularis mucosae.



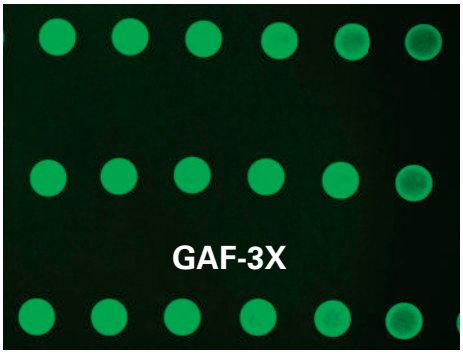
Intestine: Fluorescence of the connective tissue lining the villi and intestinal crypts, and the submucosa endothelia, honeycomb-like fluorescence in the muscular layer.

In a study with 298 paediatric coeliac disease patients (bioptically confirmed, ≥Marsh 2) and 574 controls (thereof 53 patients with chronic inflammatory bowel diseases), the EUROIMMUN Anti-Endomysium IIFT (IgA, IgG) was investigated with respect to its sensitivity and specificity with the substrates liver and oesophagus (Wolf et al., Clin Chim Acta 2016; 460: 72-77). Also here, the very high diagnostic accuracy of the EmA IgA antibody detection was confirmed and it could be shown that both tissues are equally suitable for the Anti-Endomysium IIFT (IgA and IgG).

Substrate		IIFT BIOCHIP Mosaic			
		n	IgA	n	IgG
Liver	Sensitivity	298	95.6 %	298	63.1 %
	Specificity	574	97.2 %	574	96.3 %
Oesophagus	Sensitivity	298	95.3 %	298	52.0 %
	Specificity	574	98.1 %	574	99.5 %

EUROPLUS Anti-Gliadin (GAF-3X) IIFT

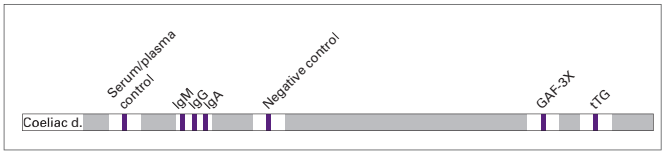
In EUROPLUS immunofluorescence tests antibody detection is performed using both tissue sections/cell substrates and monospecifically reacting antigen dots. For the EUROPLUS Anti-Gliadin (GAF-3X) IIFT, the designer antigen gliadin (GAF-3X) is coated onto BIOCHIPs in small droplets. In the case of a positive result, the circular substrate spots show a clearly visible fluorescence. The monospecific EUROPLUS substrate is offered in the form of mosaics in combination with tissue substrates for the parallel detection of CD-AGFA and EmA. When the Anti-Gliadin (GAF-3X) ELISA was used as a reference method, there was a sensitivity of 95 % for IgA (n = 122) and of 100 % for IgG (n = 114) for the EUROPLUS Anti-Gliadin (GAF-3X) IIFT. In a panel of healthy blood donors, a specificity of 99 % for IgA (n = 200) and of 100 % for IgG (n = 200) was determined.



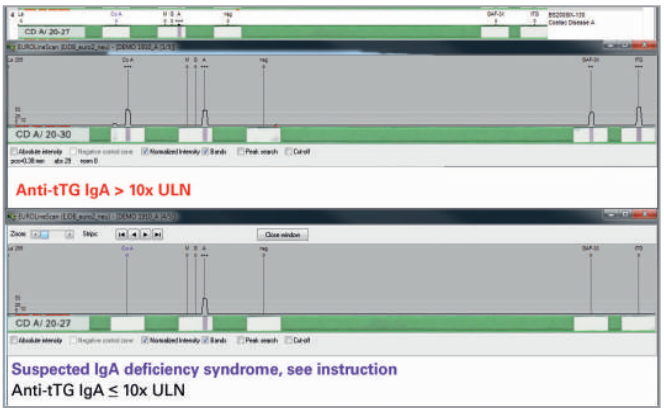
III. Immunoblot test systems for the diagnosis of coeliac disease

EUROLINE Coeliac Disease Profile (IgA, IgG)

For optimal diagnosis, detection of antibodies against both tTG and GAF-3X is recommended. The combination of tTG and GAF-3X in the EUROLINE Coeliac Disease Profile (IgA, IgG) enables simultaneous detection of reactions to both antigens.



Several serum panels which were precharacterised by CE-marked Anti-GAF-3X and Anti-tTG ELISA reference tests, respectively, were investigated for anti-GAF-3X and anti-tTG antibodies using the EUROLINE Coeliac Disease Profile. With respect to these test systems, there were sensitivities of 100 % for IgA (n = 33) and IgG (n = 44), at specificities of 88.9 % and 100 %, respectively, for the EUROLINE. For anti-tTG, there were sensitivities of 100 % for IgA (n = 44) and IgG (n = 44) at specificities of 96.9 % and 100 %, respectively, for the EUROLINE. In order to determine the reference range, a sample panel of healthy blood donors (n = 150) was investigated. All reacted correctly as negative.



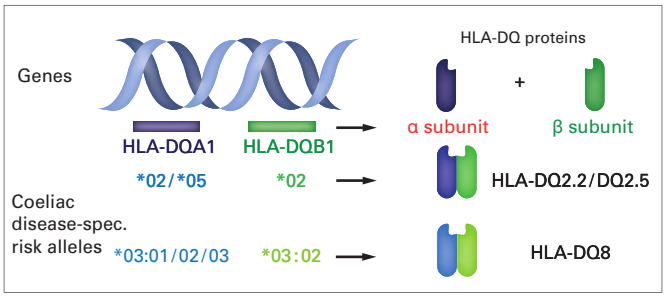
If the immunoblot strips are evaluated using the EUROLineScan software, quantitative analysis of the measurement results (determined based on the criterion "upper limit of normal", ULN) is possible. The presence of a result > 10 x ULN is automatically displayed. Moreover, the EUROLINE Coeliac Disease Profile (IgA) includes an IgA-specific serum/plasma control for detection of IgA deficiency, which occurs especially often in patients with coeliac disease. If EUROLineScan detects a positive result for the IgA conjugate control, but a negative signal for the serum/plasma control, the program issues an alert about a suspected IgA deficiency syndrome. Thereby, the risk of false negative results is effectively prevented.

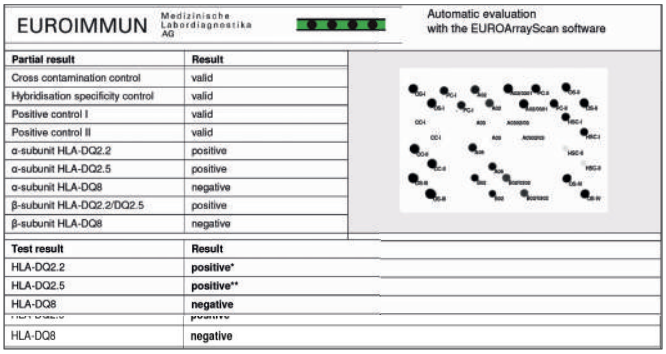
IV. Molecular genetic determination of the HLA types

HLA-DQ2/DQ8-h Direct

The EUROArray HLA-DQ2/DQ8-h Direct enables simple and fast determination of the relevant HLA-DQ types specific for coeliac disease in humans. HLA-DQ molecules are heterodimers composed of an alpha and beta subunit. The alpha and beta subunit are coded by the genes HLA-DQA1 and HLA-DQB1, respectively. In the human population there are a large number of different variants of these genes (alleles). The allele combinations which code for HLA-DQ2.2, -DQ2.5 and -DQ8 are considered risk factors for the development of coeliac disease.

The EUROArray HLA-DQ2/DQ8-h Direct detects all clinically important HLA-DQA1 and HLA-DQB1 alleles and allows for an improved risk assessment through the differentiation between homo- and heterozygous presence of the alleles coding for the alpha and beta subunits of HLA-DQ2.2 and -DQ2.5. In this way, the HLA-DQ2 and HLA-DQ8 types are clearly identified and a secure diagnosis is enabled. If neither of the two types is detected in a patient, coeliac disease may be excluded with a probability of nearly 100 % (negative predictive value at least 98 %). The test can be easily performed using EDTA blood or isolated genomic patient DNA as sample material. Evaluation, reporting, and data archiving by the system are performed objectively and automatically by means of the EUROArrayScan software.





V. Automation of coeliac disease diagnostics

All EUROIMMUN test systems for the diagnosis of coeliac disease can be automatically processed and/or evaluated.

IIFT using IF Sprinter / Sprinter XL:

- Simultaneous analysis of 96 (XL: 240) samples and 15 (XL: 30) slides
- Integrated barcode scanner for sample recognition
- Data matrix codes for automatic allocation of slides
- Visual evaluation at the fluorescence microscope, e.g. EUROStar III Plus or automated on-screen diagnosis using EUROPattern Suite

ELISA using Analyzer I or I-2P or EUROLabWorkstation ELISA:

- Analysis of up to 7 microtiter plates and 180 samples in parallel (Analyzer I), or 15 microtiter plates and >700 samples (EUROLabWorkstation ELISA)
- Autonomous sample and reagent recognition via barcodes and integrated barcode scanner
- Automatic registration of controls and calibrators

EUROLINE using EUROBlotOne:

- Fully automated evaluation of up to 44 immunoblots (EUROLINE, EUROLINE-WB, Western Blot) per run
- The evaluation is performed automatically using the EUROLineScan software, established worldwide

EUROArray using the EUROArrayScanner:

- Fully automated evaluation using the EUROArrayScanner and the corresponding software
- Autonomous data transfer and archiving

EUROIMMUN test systems in the diagnosis of coeliac disease

Secure diagnosis without biopsy

- In a study with 1071 tested samples, the Anti-tTG ELISA (IgA) contributed to a clear diagnosis. The additional determination of CD-AGFA by means of the Anti-Gliadin (GAF-3X) ELISA (IgG) further increased the statistical accuracy so that the share of tested persons requiring biopsies could be reduced to below 11 % (Wolf et al., PLOS One 2014; 9(5): e97853).
- The combination of the Anti-tTG ELISA (IgA) and the Anti-Gliadin (GAF-3X) ELISA (IgG) performed best with respect to confirmation and exclusion of coeliac disease. The first international prospective study with 898 patients showed that, observing the ESPGHAN criteria, a large number of biopsies can be prevented with this strategy (Wolf, Petroff et al., Gastroenterology 2017; 153: 410 - 419).
- The value of the diagnostic criterion "Anti-tTG IgA > 10 x ULN" was confirmed in an international multicentre study with 707 paediatric patients. The observance of the criterion prevented more than 50 % of biopsies (Werkstetter et al., Gastroenterology 2017; doi:10.1053).
- In comparison with other tests from competitors, the Anti-tTG ELISA (IgA) was able to detect the most > 10 x ULN results in a panel of 59 bioptically confirmed samples (Bufler et al., Z Gastroenterol 2015; 53: 110 - 114).

n = 59	Anti-tTG ELISA	Competitor 1	Competitor 2	Competitor 3
> 10 x ULN (IgA)	53	45	42	27

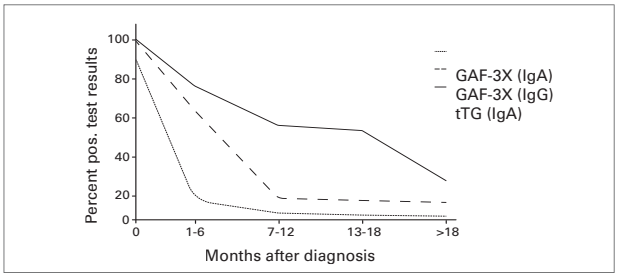
Reliable serology in patients with IgA deficiency

- The detection of GAF-3X-specific, CD-AGFA (IgG) is a useful alternative to IgA-specific tests in patients with IgA deficiency syndrome (Wolf, Petroff et al., Gastroenterology 2017; 153: 410 - 419).
- The sensitivity of the Anti-Gliadin (GAF-3X) ELISA (IgG) proved to be significantly higher compared to the sensitivity of the EmA IIFT (IgG) in a panel of 34 patients with selective immunoglobulin A deficiency (sIgAD), and exceeds the sensitivity of the Anti-tTG ELISA (IgG) (Villalta et al., Clin Chem 2010; 56. 464.468).

n = 34	Anti-GAF-3X ELISA (IgG)	Anti-tTG ELISA (IgG)	EmA IIFT (IgG)
Sensitivity	88.2 %	82.4 %	75.8 %
Specificity	97.5 %	99.9 %	99.0 %

Effective monitoring of a gluten-free diet

- A gluten-free diet (GFD) is essential for the health of patients with coeliac disease. The GFD can be easily monitored using the Anti-Gliadin (GAF-3X) ELISA and the Anti-tTG ELISA, since if the diet is adhered to, decreasing antibody concentrations can be detected (IgA, IgG).
- In an 18-month study, the majority of 78 tested patients adhering to GFD showed a significant decrease in coeliac disease-associated IgA and IgG antibodies. Moreover, in comparison to tests from other manufacturers, the EUROIMMUN Anti-tTG ELISA (IgA) reacted most sensitively to increasing titers if the dietary rules were not observed (Bufler et al., Z Gastroenterol 2015; 53: 110 - 114).



ORDERING

Test system	Substrate	Order number	Test name
ELISA	Tissue transglutaminase	EA 1910-9601 A or G	Anti-Tissue Transglutaminase ELISA
	Anti-Gliadin (GAF-3X)	EV 3011-9601 A or G	Anti-Gliadin (GAF-3X) ELISA
IIFT	Endomysium	FA 1911-1005 A or G	IIFT primate oesophagus
		FA 1913-1005 A or G	IIFT primate intestine
		FA 1914-1005 A or G	IIFT primate liver
	Gliadin (GAF-3X)	FA 1911-1005-1 A or G	EUROPLUS Gliadin (GAF-3X) with primate oesophagus
		FA 1913-1005-1 A or G	EUROPLUS Gliadin (GAF-3X) with primate intestine
		FA 1914-1005-1 A or G	EUROPLUS Gliadin (GAF-3X) with primate liver
EUROLINE	tTG / Gliadin (GAF-3X)	DL 1910-1601 A or G	EUROLINE Coeliac Disease Profile
EUROArray	DNA microarray	MN 5320-####-V	EUROArray HLA-DQ2 / DQ8-h Direct

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