EUROLINE Coeliac Disease Profile (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DL 1910-1601 G	Tissue transglutaminase and gliadin (GAF-3X)	IgG	Ag-coated immunoblot strips	16 x 01 (16)

Indications: The EUROLINE test kit provides a qualitative in vitro assay for human autoantibodies of the immunoglobulin class IgG against the two antigens **tissue transglutaminase (tTG) and gliadin (GAF-3X) (gliadin-analogue fusion peptide)** in serum or plasma for the diagnosis of gluten-sensitive enteropathy and dermatitis herpetiformis Duhring.

Application: For optimal serological diagnosis of coeliac disease, the determination of autoantibodies against tissue transglutaminase (anti-tTG) and deamidated epitopes of gliadin peptides (anti-GAF-3X) is recommended. The determination of autoantibodies of immunoglobulin classes A (IgA) and G (IgG), in the case of IgA deficiency syndrome, plays an important role.

Principles of the test: The test kit contains test strips coated with parallel lines of purified antigens. In the first reaction step, the immunoblot strips are incubated with diluted patient samples. In the case of positive samples, the specific IgG antibodies (also IgA and IgM) will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format	Symbol
1. Test strips coated with the antigens: Tissue transglutaminase and gliadin (GAF-3X)	16 strips	STRIPS
2. Positive control (IgG, human), 100x concentrate	1 x 0.02 ml	POS CONTROL 100x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	1 x 3 ml	CONJUGATE 10x
4. Sample buffer ready for use	1 x 100 ml	SAMPLE BUFFER
5. Wash buffer 10x concentrate	1 x 50 ml	WASH BUFFER 10x
6. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	SUBSTRATE
7. Incubation tray	2 x 8 channels	
8. Test instruction	1 booklet	

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

Storage and stability: The test kit must be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9899-0130 Incubation tray with 30 channels
ZD 9898-0130 Incubation tray with 30 channels (black, for the EUROBlotCamera system)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and the EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a visual evaluation is to be performed in individual cases, the required evaluation protocol can be ordered under:
ZD 1910-0101 Visual evaluation protocol EUROLINE Coeliac Disease Profile.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips the package should be sealed tightly and stored at +2°C to +8°C.

- **Positive control:** The control is a 100x concentrate. For the preparation of the ready for use control the amount required should be removed from the bottle using a clean pipette and diluted 1:101 with sample buffer. Example: add 15 µl of control to 1.5 ml of sample buffer and mix thoroughly. The ready for use diluted control should be used at the same working day.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with sample buffer. For one test strip, dilute 0.15 ml enzyme conjugate with 1.35 ml sample buffer. The ready for use diluted enzyme conjugate should be used at the same working day.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is supplied as a 10x concentrate. For the preparation of the ready for use wash buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with distilled water. For one test strip, dilute 1 ml in 9 ml of deionised or distilled water. The ready for use diluted wash buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

Warning: The control of human origin has tested negative for HBsAg, and antibodies against anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:101** with sample buffer. For example, add 15 µl of sample to 1.5 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

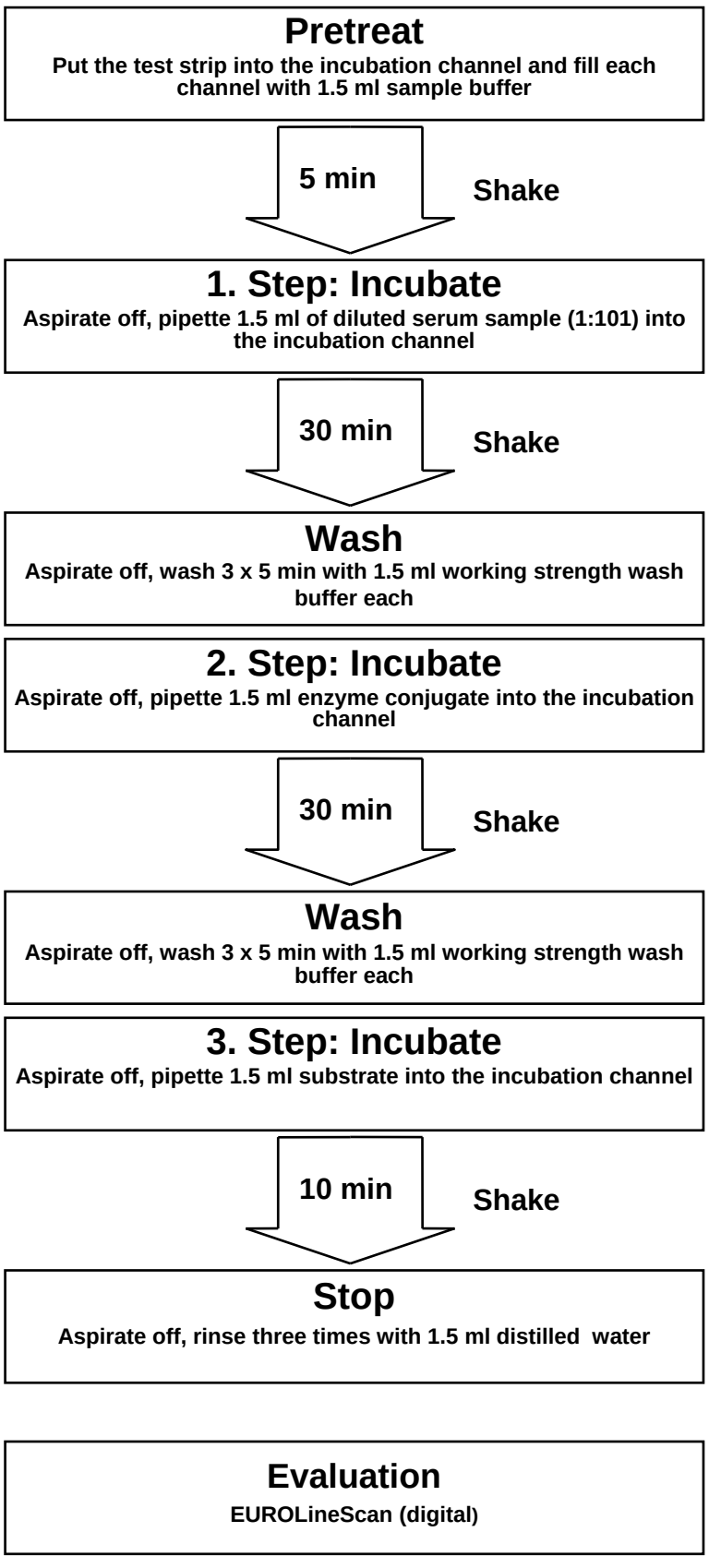
Pretreat:	Remove the required amount of test strips from the package and place them each in an empty channel. The number on the test strip should be visible. Fill the channels of the incubation tray according to the number of serum samples that should be tested with 1.5 ml sample buffer each. Incubate for **5 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Incubate: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples and incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength wash buffer on a rocking shaker.
Incubate: (2nd step)	Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-labelled anti-human IgG) into each channel and incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Incubate: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stop:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.
Evaluate:	Place test strip on the evaluation protocol, air dry and evaluate.

For automated incubation with the **EUROBlotMaster** select the program **Euro01 AAb EL30-V2.**

For automated incubation and evaluation with the **EUROBlotOne** select the program **EURO 16**.

EUROLINE Coeliac Disease Profile (IgG)

Incubation protocol



Interpretation results

Handling: For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **test** into EUROLineScan is **Coeliac Disease G**.

If a visual evaluation must be performed, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 1910-0101.

Note: Correct performance of the incubation is indicated by an intense staining of the serum/plasma control band.

Antigens and their arrangement on the strips: The EUROLINE test strips have been coated with the following antigens:

Antigens:

tTG: Recombinant tissue transglutaminase

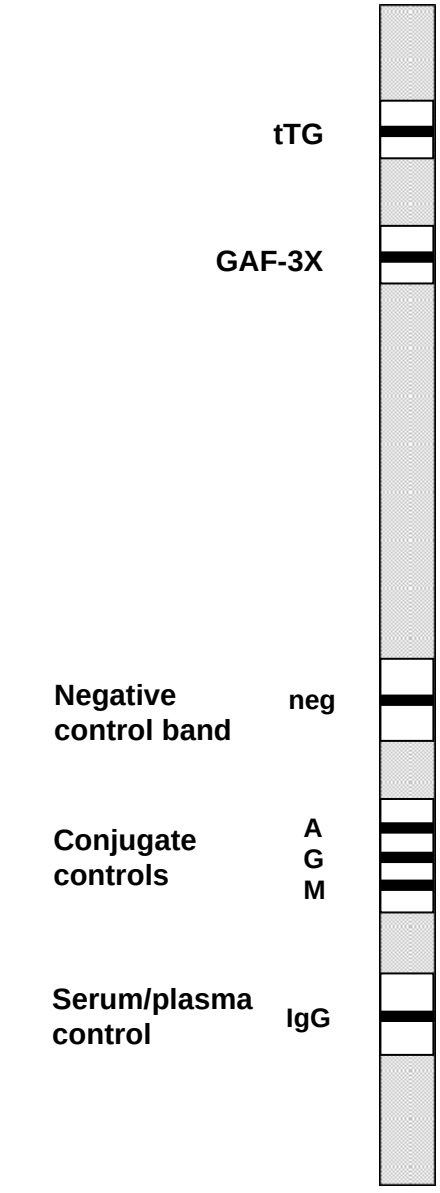
GAF-3X: Recombinant GAF-3X (gliadin-analogue fusion peptide)

Control bands:

Negative control bands: Contains components from the expression system which are used for antigen purification. In the case of a positive band, an unspecific reaction cannot be excluded.

Conjugate controls:
Controls to confirm that the conjugate was correctly used.

Serum/Plasma control:
Control to confirm that the incubation was conducted correctly.



EUROIMMUN recommends interpreting results based on the signal intensity:

Signal **Visual evaluation**	**Signal intensity** **EUROLineScan** **Flatbed scanner**	**Result**	
No signal	0-5	o	Negative
Very weak band	6-10	(+)	Borderline
Medium to strong band	11-25 or 26-50	+, ++	Positive
Very strong band with an intensity comparable to the control band	>50	+++	Strong positive

Results in the **borderline range** (+) should be evaluated as increased but negative. The table above contains **values** for the evaluation using a flatbed scanner. The **values** for other instruments supported by EUROLineScan can be found in the EUROLineScan program. To do so mark the corresponding assay in the test list ("Help" → "Test") and click on details and select **the corresponding instrument** in **"image source"**.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided.

Cross reactions: The quality of the antigen substrates used (antigen and antigen source) ensures the high analytical specificity of the test system. The EUROLINE specifically enables IgG antibodies against **tissue transglutaminase and gliadin (GAF-3X)**. Cross reactions of other antibodies were not detected.

Interference: Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml for haemoglobin, of 20 mg/ml for triglycerides and of 0.4 mg/ml bilirubin showed no effect on the analytical results of the present EUROLINE.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Sensitivity and specificity:
46 serum samples (precharacterised with a CE-notified reference test) were investigated for autoantibodies against gliadin (GAF-3X) (borderline results were not included in the calculation).

n=46		**ELISA Anti-Gliadin (GAF-3X) IgG**		
		positive	borderline	negative
EUROLINE Coeliac Disease Profile (IgG)	positive	8	0	1
	borderline	3	0	1
	negative	1	0	32

In the investigated panel, a sensitivity of 88.9% at a specificity of 97.0% was determined with respect to the reference system.

44 serum samples (precharacterised with a CE-notified reference test) were investigated for autoantibodies against tTG (borderline results were not included in the calculation.

n=44		ELISA Anti-tTG IgG		
		positive	borderline	negative
EUROLINE Coeliac Disease Profile (IgG)	positive	10	0	1
	borderline	0	0	2
	negative	0	0	31

In the investigated panel, a sensitivity of 100.0% at a specificity of 96.9% was determined with respect to the reference system.

Other studies: The prevalence of coeliac disease specific autoantibodies was investigated in patients with SLE, RA, diabetes or Crohn's disease.

Patient panel	n (73)	Anti-GAF-3X IgG positive	Anti-tTG IgG positive
SLE	15	0.0%	0.0%
RA	20	0.0%	0.0%
Diabetes	18	5.6%	0.0%
Morbus Crohn	20	0.0%	0.0%

Reference range: The reference range was determined in a sample panel of healthy blood donors (n=150). All blood donors reacted negative.

Clinical significance

The serological determination of disease-specific antibodies against endomysium and gliadin (IgA and IgG) is an essential component in the diagnosis of gluten-sensitive enteropathy (coeliac disease, sprue) and is suited for evaluation of disease course and therapy success.

Coeliac disease is a systemic autoimmune disease with genetic predisposition which shows in affected individuals as a reaction to the consumption of gluten. Gluten is found in various cereals (e.g. wheat, barley, rye). The most important protein to trigger coeliac disease is gliadin.

In Europe, the estimated prevalence of coeliac disease amounts to approximately 1%. Unspecific or mild symptoms may lead, however, to a large number of further cases which are not diagnosed. Alongside the typical inflammation of the mucous membrane of the small intestine, the clinical picture comprises also symptoms such as tiredness, borborygmus, abdominal pain and diarrhoea, as well as weight loss, anaemia, fertility disorders, growth retardation and osteoporosis as a consequence of nutrient malabsorption. Some patients with coeliac disease also suffer from Duhring's disease, a chronic skin disease accompanied by blister formation. Neurological manifestations such as gluten ataxia are also comprised in the atypical symptoms of coeliac disease.

Coeliac disease is caused by both genetic and environmental factors. The gliadin which is consumed with food can only be partially digested in the intestine. In patients with coeliac disease, the remaining gliadin peptides may pass through the epithelium of the small intestine and enter the underlying connective tissue. There, the protein fragments are deaminated by the enzyme tissue transglutaminase (tTG). In this process, the amino acid glutamine is converted into glutamic acid. If there is a genetic predisposition (human leukocyte antigens (HLA)-DQ2 or DQ8), these modified peptides are increasedly presented to the immune system by antigen presenting cells. As a consequence, proinflammatory cytokines are secreted and antibodies against both specific, deamidated gliadin epitopes and the body-own enzyme tTG are produced. The immune response leads to inflammation and damage to the mucous membrane of the small intestine which is histologically characterised by an atrophic villi structure and hyperplastic intestinal crypts. The Marsh classification distinguishes three types of coeliac disease, according to the severity of this intestinal histopathology:

Marsh type I: increase in intraepithelial lymphocytes (>40 IEL/100 epithelial cells) with normal mucous membrane architecture
Marsh type II: additional crypt hyperplasia with normal villi
Marsh type III: IEL increase, crypt hyperplasia, degeneration of epithelial cells and villous atrophy. Type III is further divided into Marsh IIIA (partial villous atrophy), Marsh IIIB (subtotal villous atrophy) and Marsh IIIC (complete villous atrophy).

Serological diagnosis is an economical and non-invasive method and significantly aids the diagnosis and monitoring of coeliac disease. In some cases, it may eliminate the necessity of a biopsy of the small intestine (see graphic).

Particularly IgA antibodies against tTG or endomysium are considered the most specific and sensitive indicator of coeliac disease. Determination of anti-tTG antibodies (IgA and IgG) is performed using a monospecific test system. Antibodies against endomysium (EmA) can be determined by IIFT, using tissue sections of oesophagus (monkey), intestine (monkey) or liver (monkey). The new antigen substrate gliadin GAF-3X was developed for the detection of antibodies (IgA, IgG) against the relevant, deaminated gliadin epitopes. In a direct comparison, the Anti-Gliadin (GAF-3X) ELISA (IgA, IgG) showed a much higher sensitivity and specificity than an anti-native gliadin ELISA (with a native gliadin substrate; anti-ngliadin ELISA). The designer antigen is a recombinant, gliadin-analogue fusion peptide (GAF) which is triplicated (3X). The fusion peptide consists of two units which are characterised on the one hand by an especially high reactivity with different sera of coeliac disease patients and on the other hand by a presumed pathophysiological relevance. Together, they only account for approximately 10% of the total gliadin. The largest part of the native protein is actually not relevant for the disease and presents immunological ballast which is mostly a target of unspecific reactions. In the most up-to-date test systems this portion is left out, resulting in a huge increase in specificity. Especially in cases of selective IgA deficiency, which is often present in coeliac disease patients and where neither Anti-tTG-ELISA IgA, nor Anti-EmA-IFT IgA lead to meaningful results, the Anti-Gliadin (GAF-3X) ELISA IgG has proven to be an excellent alternative. The test is equally suitable for diagnosis of coeliac disease in children under 2 years. Different studies have shown that ELISAs for the detection of IgG antibodies against deaminated gliadin epitopes, such as the Anti-Gliadin (GAF-3X) ELISA IgG, and the Anti-tTG ELISA IgA, obtain a higher diagnostic accuracy than ELISAs with a native gliadin substrate (IgA, IgG). Therefore, they should be more preferably used in comparison to the anti-ngliadin ELISAs in paediatric coeliac disease diagnostics, independently from the age of the patient.

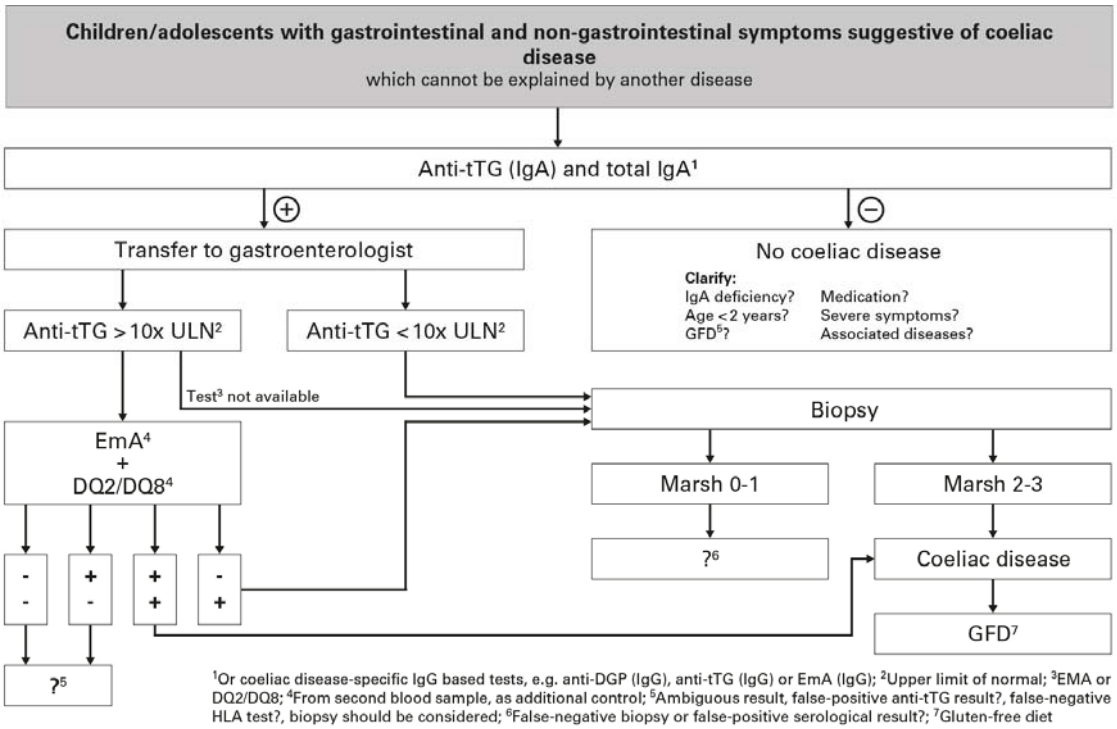
Apart from the use in the Anti-Gliadin (GAF-3X) ELISA (IgA, IgG), the designer antigen GAF-3X is also available as an individual antigen (antigen dots) for IIFT in a BIOCHIP combination (EUROPLUS) with tissue sections, and in the EUROLINE Coeliac Disease Profile.

HLA typing is another important component of coeliac disease diagnostics, especially in cases of unclear biopsy findings, unclear serological findings or asymptomatic patients with increased disease risk (see graphic). With a negative predictive value from the risk factors HLA-DQ2 and DQ8 of at least 98%, coeliac disease can be virtually excluded if neither of the alleles is detected in the patient. The EUROArray HLA-DQ2/DQ8 provides easy and reliable determination of all disease-associated alleles.

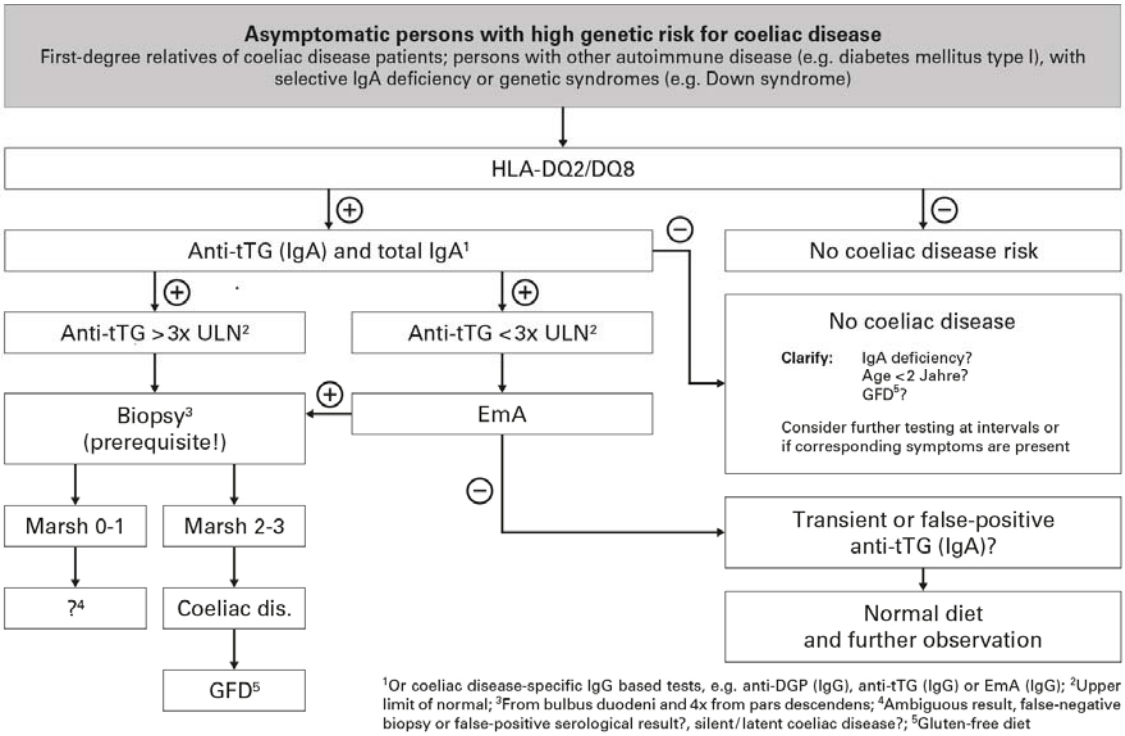
The antigen-antibody reactions also provide useful information for the **follow-up and monitoring of a gluten-free diet**. Decreasing antibody titers indicate success in treatment and usually go together with an improvement of the clinical symptoms.

An overview of the new European **ESPGHAN** (**E**uropean **S**ociety for **P**aediatric **G**astroenterology **H**epatology **a**nd **N**utrition) guidelines for the diagnosis of coeliac disease in children and adolescents was published in 2012. These guidelines distinguish in the diagnostic procedure between patients whose clinical symptoms indicate coeliac disease, and asymptomatic patients who have an increased risk of coeliac disease.

1. Children/adolescents with gastrointestinal and non-gastrointestinal symptoms suggestive of coeliac disease which cannot be explained by any other disease



2. Persons without specific symptoms (with genetic risk for coeliac disease, increased risk for coeliac diseases e.g. in first-degree relatives of coeliac disease patients, persons with other autoimmune diseases (e.g. diabetes mellitus type I), with selective IgA deficiency or genetic syndromes (e.g. Down syndrome))



Literature references

1. Fasano A. **Celiac disease - how to handle a clinical chameleon.** N Engl J Med (2003) 348:25.
2. Felber J, Aust D, Baas S, Bischoff S, Bläker H, Daum S, Keller R, Koletzko S, Laass M, Nothacker M, Roeb E, Schuppan D, Stallmach A. **Ergebnisse einer S2k-Konsensuskonferenz der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselerkrankungen (DGVS) gemeinsam mit der Deutschen Zöliakie-Gesellschaft (DZG) zur Zöliakie, Weizenallergie und Weizensensitivität.** Z Gastroenterol (2014) 52: 711-743.
3. Husby S., Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP; ESPGHAN Working Group on Coeliac Disease Diagnosis; ESPGHAN Gastroenterology Committee; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. **European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease.** J Pediatr Gastroenterol Nutr 54 (2012) 136-160.
4. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, Murray L, Metzger MH, Gasparin M, Bravi E, Mäki M; Coeliac EU Cluster, Project Epidemiology. **The prevalence of celiac disease in Europe: results of a centralized, international mass screening project.** Ann Med (2010) 42(8):587-95.
5. Prause C, Richter T, Koletzko S, Uhlig HH, Hauer AC, Stern M, Zimmer K-P, Laass MW, Probst C, Schlumberger W, Mothes T. **New developments in serodiagnosis of childhood celiac disease. Assay of antibodies against deamidated gliadin.** Ann N Y Acad Sci (2009) 1173: 28-35.
6. Prause C, Ritter M, Probst C, Daehnrich C, Schlumberger W, Komorowski L, Lieske R, Richter T, Hauer AC, Stern M, Uhlig HH, Laass MW, Zimmer K-P, Mothes T. **Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease.** J Pediatr Gastroenterol Nutr (2009) 49(1): 52-58.
7. Richter T, Bossuyt X, Vermeersch P, Uhlig HH, Stern M, Hauer A, Zimmer K-P, Mearin L, Roo de JHC, Dähnrich C, Mothes T. **Determination of igg and iga antibodies against native gliadin is not helpful for the diagnosis of coeliac disease in children up to 2 years old.** J Pediatr Gastroenterol Nutr (2012) 55(1): 21-25.
8. Schwertz E, Kahlenberg F, Sack U, Richter T, Stern M, Conrad K, Zimmer K-P, Mothes T. **Serologic assay based on gliadin-realted nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease.** Clin Chem (2004) 50(12): 2370-2375.
9. Villalta D,Tonutti E, Prause C, Koletzko S, Uhlig HH, Vermeersch P, Bossuyt X, Stern M, Laass MW, Ellis JH, Ciclitira PJ, Richter T, Daehnrich C, Schlumberger W, Mothes T. **IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with iga deficiency.** Clin Chem (2010) 56:3.
10. Wolf J, Hasenclever D, Petroff D, Richter T, Uhlig HH, Laaß MW, Hauer A, Stern M, Bossuyt X, Laffolie de J, Flemming G, Villalta D, Schlumberger W, Mothes T. **Antibodies in the diagnosis of coeliac disease: a biopsy-controlled, international, multicentre study of 376 children with coeliac disease and 695 controls.** PLoS ONE (2014) 9(5): e97853. doi:10.1371/journal.pone.0097853.