



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 (Dühring'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:

Panel	n	Gliadin (GAF-3X) (IgA) positive	Gliadin (GAF-3X) (IgG) positive	Gliadin (IgA) positive	Gliadin (IgG) positive	tTG (IgA) positive	tTG (IgG) positive
Coeliac disease (children >1.25 years) ¹	139	115	118	96	127	135	45
Dühring's dermatitis herpetiformis (adults, diet unknown) ¹	13	9	5	10	7	9	1
Sensitivity	139	82.7%	84.9%	69.1%	91.4%	97.1%	32.4%
Gastroenteropathies, negative biopsy for coeliac disease ¹	129	6	4	8	36	10	0
Chronic-inflammatory bowel disease, negative biopsy for coeliac disease ¹	16	1	0	1	6	0	0
Specificity	145	95.2%	97.2%	93.8%	71.0%	93.1%	100.0%
Sensitivity at 95% specificity	284	84.9%	94.2%	61.8%	33.1%	96.4%	64.0%
Rheumatoid arthritis ²	200	6	1	31	19	1	0
Sjögren's syndrome ²	200	9	4	29	11	3	0
Systemic lupus erythematosus ²	100	3	7	23	21	0	0
Progressive systemic sclerosis ²	126	6	3	18	5	4	1
Specificity	771	96.0%	97.5%	85.7%	87.3%	97.7%	99.9%

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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.

EUROIMMUN Microplate ELISA

- Autoantibody determination:**
- AMA M2-3E (IgG)
 - ANCA Profile (IgG)
 - ANA Screen (IgG)
 - ANA Screen 9* or 11* (IgG)
 - ANA VarioProfile (IgG)
 - BP180-4X (IgG)
 - C1q (IgG)
 - cardiolipin (IgA, IgG, IgM, IgAGM)
 - circulating immune complexes (CIC)
 - cyclic citrullinated peptide (CCP; IgG)
 - centromere protein B (IgG)
 - double-stranded DNA (dsDNA, nDNA; IgG)
 - dsDNA-NcX (IgG)
 - ENA Pool* (IgG)
 - ENA PoolPlus (IgG)
 - ENA ProfilePlus 1 or 2 (IgG)
 - ENA SLE Profile 1 or 2 (IgG)
 - GAD
 - GAD/IA-2 Pool
 - glomerular basement membrane (GBM; IgG)
 - β2-glycoprotein 1 (IgA, IgG, IgM, IgAGM)
 - histones (IgG)
 - IA-2
 - intrinsic factor (IgG)
 - Jo-1 (IgG)
 - liver cytosolic antigen type 1 (LC-1; IgG)
 - liver-kidney microsomes (LKM-1; IgG)
 - myeloperoxidase (MPO; IgG)
 - nRNP/Sm (IgG)
 - nucleosomes (IgG)
 - p53 (IgG)
 - parietal cells (PCA; IgG)
 - PM-Scl (PM-1; IgG)
 - phosphatidylserine (IgA, IgG, IgM, IgAGM)
 - proteinase 3 (IgG)
 - PR3 hn-hr (IgG)
 - PR3 capture (IgG)
 - rheumatoid factor (IgA, IgG, IgM)
 - ribosomal P-proteins (IgG)
 - Scl-70 (IgG)
 - single-stranded DNA (ssDNA; IgG)
 - SLA/LP (IgG)
 - Sm (IgG)
 - SS-A (Ro; IgG)
 - SS-B (La; IgG)
 - thyroglobulin (TG; IgG)
 - thyroid peroxidase (TPO; IgG)
 - tissue transglutaminase (endomy.; IgA, IgG)
 - TSH receptor (TBI; IgG)
 - TRAk Fast (IgG)

- Further autoimmune diagnostics:**
- GAF-3X (IgA, IgG)
 - gliadin (IgA, IgG)
 - Saccharomyces cerevisiae (IgA, IgG)

- Infectious serology:**
- Adenovirus (IgA, IgG, IgM)
 - Borrelia (IgG, IgM)
 - Borrelia VlsE (IgG)
 - Chlamydia pneumoniae (IgA, IgG, IgM)
 - Chlamydia trachomatis (IgA, IgG, IgM)
 - Cytomegalovirus (IgG, IgM)
 - Diphtheria toxoid (IgG)
 - Epstein-Barr virus capsid ag (IgA, IgG, IgM)
 - Epstein-Barr virus early ag (IgA, IgG, IgM)
 - Epstein-Barr virus nuclear ag, EBNA-1 (IgG)
 - Helicobacter pylori (IgA, IgG)
 - Helicobacter pylori CagA (IgA, IgG)
 - HSV-1 glycoprotein C1; IgA, IgG, IgM)
 - HSV-2 glycoprotein G2; IgA, IgG, IgM)
 - HSV-1/2 Pool (IgA, IgG, IgM)
 - Influenza virus type A (IgA, IgG, IgM)
 - Influenza virus type B (IgA, IgG, IgM)
 - Legionella pneumophila (IgA, IgG, IgM)
 - Measles virus (IgG, IgM)
 - Mumps virus (IgG, IgM)
 - Mycoplasma pneumoniae (IgA, IgG, IgM)
 - Parainfluenza virus Pool (IgA, IgG, IgM)
 - RSV (IgA, IgG, IgM)
 - Rubella virus (IgG, IgM)
 - SARS-CoV (IgG)
 - TBE virus (IgG, IgM)
 - Tetanus toxoid (IgG)
 - Toxoplasma gondii (IgG, IgM)
 - Treponema pallidum (IgG, IgM)
 - Varicella zoster virus (IgG, IgM)
 - Yersinia enterocol. virulence fact. (IgA, IgG)

- Allergology:**
- total IgE
 - Allercoat™ 6-ELISA (600 different allergens and allergen mixtures)

- Serum proteins and tumour markers:**
- anti-p53

* Currently not available as IVD in the EU.

Made in Germany



EUROIMMUN Immunoblots

Autoantibody determination:

EUROASSAY:

flexible profiles of up to 7 antigens from:

ENA and related antigens: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2

liver antigens: LKM-1, LC-1, SLA/LP, AMA M2, M4, M9

ANCA antigens: MPO, PR3

thyroid antigens: TG, TPO

EUROLINE:

ANA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B, dsDNA, nucleosomes, histones, ribosomal P-proteins

ANA Profile 3: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2

Anti-ENA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1

Myositis Profile: Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12, Ro-52

Liver Profiles: AMA M2, 3E (BPO), Sp100, PML, gp210, LKM-1, LC-1, SLA/LP, Ro-52

Neuronal Antigens Profile: amphiphysin, CV2/CRMP5, PNM2A (Ma-2), Ri, Yo, Hu

Anti-Ganglioside Profile 1: GM1, GD1b, GQ1b

Anti-Ganglioside Profile 2: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b

ANCA Profiles: MPO, PR3, GBM

EUROLINE-WB:

liver-specific antigens (+ recomb. SLA/LP)

neuronal antigens (+ recomb. Hu, Yo, Ri)

HEP-2 cell antigens (+ SS-A and Ro-52, CENP B)

Myositis ag (Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12)

Infectious serology:

EUROLINE:

EBV Profile (IgG, IgM, VCA gp125, VCA p19 and EBNA-1, p22, EA-D)

TORCH Profile* (T. gond., rubella, CMV, HSV-1, -2)

Malaria Profile 1: Plasmodium falciparum HRP-2 and MSP-2, Plasmodium vivax MSP and CSP

Westemblot:

Borrelia burgdorferi (IgG, IgM)

Borrelia afzelii (IgG, IgM)

Borrelia garinii (IgG, IgM)

Epstein-Barr virus (IgG, IgM)

Helicobacter pylori (IgA, IgG)

Treponema pallidum (IgG, IgM)

Yersinia enterocol. virulence fact. (IgA, IgG)

EUROLINE-WB:

Anti-Borrelia (B. afzelii + rec. VlsE)

Anti-HSV (HSV-1 + HSV-2 gG2)

Treponema pallidum + cardiolipin

Allergology:

EUROASSAY:

Domestic Animal Profile (IgE)

Food Profile (IgE)

Inhalation Profile (IgE)

Insect Venom Profile (IgE)

Latex Profile (IgE)

Latex plus Profile (with ficus and fruit; IgE)

EUROLINE:

Atopy Profile (IgE)

Food Profile (IgE)

Inhalation Profile (IgE)

Paediatric Inhalation Profile

Pollen-Food Cross Reaction Profile (IgE)

Software/Automation:

EUROLineScan

camera system EUROBlotCamera

scanner system EUROBlotScanner

incubation processor EUROBlotMaster

EUROIMMUN

Radioimmunoassays

Autoantibody determination:

thyroid peroxidase (TPO; IgG)

thyroglobulin (TG; IgG)

TSH receptor (IgG)

acetylcholine receptor (AChR; IgG)

glutamic acid decarboxylase (GAD; IgG)

insulin (IAA; IgG)

P/Q calcium channel* (VGCC; IgG)

tyrosine phosphatase (IA2; IgG)

dsDNA (IgA/IgG/IgM)

Antigen determination:

thyroglobulin (TG)

Hormone determination:

free triiodothyronin (FT3)

free thyroxin (FT4)

thyrotropin (TSH)

calcitonin

* Currently not available as IVD in the EU.

Made in Germany

Version: 10/07

EA_3011_D_UK_A01

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, R² 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).

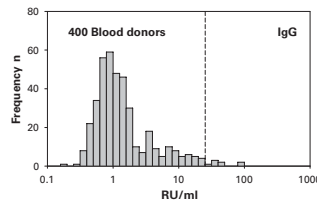
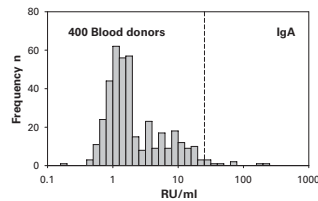
Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs. The mean value of 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 gastroenteropathies, 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:

Coeliac disease, n=139	Gliadin (GAF-3X) (IgA)	
	positive	negative
Gliadin (IgA)	93	3
	22	21
Gliadin (IgG)	Gliadin (GAF-3X) (IgG)	
	positive	negative
	113	14
	5	7

Panels, n=145	Gliadin (GAF-3X) (IgA)	
	positive	negative
Gliadin (IgA)	3	6
	4	132
Gliadin (IgG)	Gliadin (GAF-3X) (IgG)	
	positive	negative
	3	39
	1	102

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).



n=400 Blood donors			
IgA			
Percentile	95.	98.	99.
Cut-off	17.1 RU/ml	26.3 RU/ml	41.2 RU/ml
IgG			
Percentile	95.	98.	99.
Cut-off	13.8 RU/ml	24.5 RU/ml	39.6 RU/ml

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):

Ig type	Specificity	Sensitivity	Cut-off
IgA	95%	86%	20.7 RU/ml
	98%	75%	53.3 RU/ml
IgG	95%	95%	11.2 RU/ml
	98%	84%	27.9 RU/ml

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)



Antibodies against gliadin in the diagnosis of coeliac disease and dermatitis herpetiformis: Important information for users of the new Anti-Gliadin(GAF-3X) ELISA.

The Anti-Gliadin(GAF-3X) ELISA is used for the determination of antibodies against gliadin in the serological diagnosis of gluten-sensitive enteropathy (coeliac disease, non-tropical sprue) and dermatitis herpetiformis Dühring. At the core of this new development is a state-of-the-art "designer antigen", from which the immunological reactive surface is created. It is a recombinant "gliadin-analogue fusion peptide", which produces a positive reaction almost exclusively in patients with coeliac disease and DHD, but not in healthy persons or patients with other gastrointestinal diseases (Schwartz, Mothes et al. 2004, Clin. Chem. 50:2370, further developed by EUROIMMUN: Probst et al. 2007, DE-OS 10 2007 025 291.0).

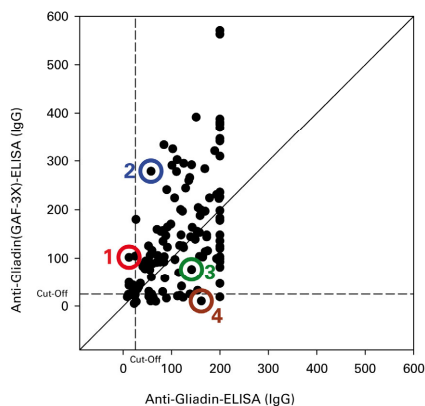
Special characteristics of the new antigen substrate

The fusion peptide consists of two components: an artificial gliadin-fragment analogue nonapeptide, which has been empirically selected from thousands of artificial variants with respect to its reactivity with coeliac disease sera; and a nonapeptide section of digested gliadin that has been deaminated by transglutaminase (glutamine to glutamic acid). This section is probably pathophysiologically relevant for coeliac disease and makes up no more than 2% of the total size of gliadin. The remaining 98% of the gliadin molecule is not used in the ELISA – immunological ballast, which serves predominantly as a target for unspecific reactions. This explains the enormous increase in specificity for the Anti-Gliadin-(GAF-3X) ELISA. Moreover, the construct is expressed in trimer form in order to increase the responsiveness of the test system.

Comparison of results

In a coeliac disease panel from Prause et al. (10th Workshop on Autoantibodies and Autoimmunity, Guadalajara, Mexico, 2008) the Anti-Gliadin(GAF-3X) ELISA showed a sensitivity of 83% (IgA) or 95% (IgG), each at 95% specificity (conventional anti-gliadin ELISA: 54% for IgA and 31% for IgG). In a group of patients with dermatitis herpetiformis Dühring (n = 36) the test yielded a sensitivity of 83% (IgA) or 78% (IgG) and was therefore almost 30% more sensitive than a conventional anti-gliadin ELISA (sensitivity IgA: 55%, IgG: 50%).

The determination of IgG antibodies against whole gliadin with conventional tests is useless for the diagnosis of coeliac disease, since a quarter of the normal population reacts positively in IgG. So up until now clinicians had to rely only on IgA, thereby missing patients with selective IgA deficiency – a disposition which is associated with coeliac disease with an above average frequency. With the Anti-Gliadin(GAF-3X) ELISA it is generally sufficient to investigate only immunoglobulin class IgG; this way coeliac disease patients with IgA deficiency are also detected.



Serum	Measurement values for the concentration of anti-gliadin AAb (IgG)	
	Conventional Anti-Gliadin ELISA	Anti-Gliadin (GAF-3X) ELISA
1	5 RU/ml	100 RU/ml
2	65 RU/ml	290 RU/ml
3	145 RU/ml	90 RU/ml
4	165 RU/ml	10 RU/ml

Four examples

Consequences for laboratory daily routine

In comparison to a conventional test system there were numerous deviations with the new Anti-Gliadin(GAF-3X) ELISA in the panel from Prause et al:

- Due to the increase in sensitivity many previously negative sera reacted positively (example 1) or higher measurement values were obtained (example 2). The percentage increase in values sometimes showed large differences from serum to serum.
- Due to the increase in specificity there was in some cases a decrease in the measurement value (example 3), or previously positive sera reacted negatively in the new test system (example 4).

We recommend that you inform your customers about the change of test in writing. We are happy to provide data sheets for this purpose. If you wish you can use the following text, in whole or in part: " For the serological diagnosis of gluten-sensitive enteropathy (coeliac disease, non-tropical sprue) and dermatitis herpetiformis Dühring we will in the future be using a new test system with higher sensitivity and specificity, the EUROIMMUN Anti-Gliadin(GAF-3X) ELISA. A purely technically caused increase or decrease in measurement values can occur, which should not be interpreted as an augmentation or decline in the clinical activity. The deviation can vary in strength from case to case".