INSTRUCTION MANUAL

REF 4045

February 14, 2008

CeliAK EmA human IgG

- 96 determinations -

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to human endomysial autoantigens in human serum

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

CeliAK EmA human IgG is used for the quantitative determination of IgG autoantibodies to human endomysial autoantigens (EmA).

Several autoantigens have been discussed for celiac disease and Dermatitis herpetiformis recently. Dieterich et al. have described two non-collagenous proteins with an apparent molecular weight of 90 and 300 kDa being the main autoantigens detected by EmA (1). Celiac disease, or gluten-sensitivity, is found already in neonates and is characterized by small intestinal damages leading to a so-called “flat” mucosa. Due to this extensive lesions mal-absorption occurs frequently accompanied with a depletion of key nutrients.

Incidence rates for celiac disease range from 1 in 300 (Western Ireland) to 1 in 4700 in European countries. However, a high number of subclinical cases of celiac disease have been detected by in-vitro tests revealing a prevalence of 4 in 1000. Individuals suffering from prolonged celiac disease additionally face an elevated risk of developing T cell lymphoma. Dermatitis herpetiformis is an autoimmune chronic relapsing skin disease characterized by formation of sub-epidermal blisters. Deposits of IgG antibodies are found in the basal membrane associated with neutrophil and eosinophil infiltration. Dermatitis herpetiformis seems to demonstrate celiac like changes in the intestine resembling the inflammatory process in celiac patients.

Diagnosis of celiac disease and Dermatitis herpetiformis comprises small intestine biopsy demonstrating a “flat” mucosa prior to a gluten-free diet and the following reconstitution of the mucosa after onset of the diet. Determination of anti-gliadin IgG and IgA by ELISA as well as the detection of EmA IgA and recently endomysial IgG (EmG) have been considered as the main serological parameters for celiac disease so far. The determination of IgG autoantibodies to endomysial antigens expand the diagnostic range of serologic parameters for the diagnosis of celiac diseases.

IgA deficient celiac patients may demonstrate EmG only. Therefore, endomysial IgG autoantibodies are the only serological parameter for celiac disease detectable in such patients apart from gliadin IgG antibodies.

GENERIC ASSAYS` range of diagnostic parameters for these two disorders includes also two kits for the determination of gliadin antibodies **Anti-Gliadin IgG** and **Anti-Gliadin IgA**. These kits employ both congruent assay schemes and the same predilution of sera allowing parallel determination with the novel **CeliAK EmA human** and **CeliAK EmA human IgG**.

(1) Dieterich et al. Gut (1995) A76-A77

PRINCIPLE OF THE TEST

CeliAK EmA human IgG is an enzyme immunoassay for the quantitative determination of IgG autoantibodies to the supposed major autoantigens in Dermatitis herpetiformis and celiac disease in human serum.

Autoantibodies of the diluted patient samples and standards react with the immobilized human autoantigens on the solid phase of the microtiter plate. CeliAK EmA human IgG guarantees the specific binding of autoantibodies usually found by immunofluorescence on endomysial antigens. Following an incubation period of 60 min at room temperature (18…25°C), unbound serum components are removed by a wash step.

The bound autoantibodies react specifically with anti-human-IgG-antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at room temperature. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3’,5,5’-tetramethyl-benzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the concentrations of the antibodies of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method is also possible.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.***

The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with purified human EmA autoantigen and gliadin fragments	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted serum) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- pipette tips
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- tubes (2 ml) for sample preparation
- distilled or de-ionized water

Size and storage

CeliAK EmA human IgG has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the CeliAK EmA human IgG have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable up to 30 days at 2 - 8 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently, avoid foam.
2. Dispense
   100 µl calibrators 0 - 4 (quantitative) or
   100 µl of calibrator 1 (semi-quantitative)

   100 µl positive control
   100 µl diluted patient samples
   into the respective wells.
3. Seal plate, incubate **60 min** at room temperature.
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at room temperature.
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min** protected from light at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

CeliAK EmA human IgG allows both the quantitative (5 calibrators) and semi-quantitative (calibrator 1 as cut-off calibrator) evaluation of the results.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective concentrations on the abscissa, x-axis, (log. scale). Anti- IgG concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results can be calculated semi-quantitatively using the binding index BI (ratio) between the optical density of an unknown sample and the optical density of **calibrator 1** (10 U/ml):

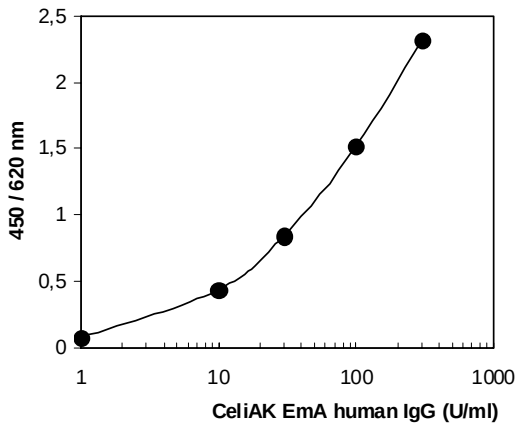
$$BI = OD_{Sample} / (OD_{Calibrator\ 1} \times 1{,}5)$$

Both evaluation variants of CeliAK EmA human IgG may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of typical assay results

well	OD (a)	OD (b)	OD (mean)	U/ml
Cal **0**	0.072	0.084	0.078	**1**
Cal **1**	0.425	0.450	0.438	**10**
Cal **2**	0.825	0.859	0.842	**30**
Cal **3**	1.501	1.530	1.516	**100**
Cal **4**	2.308	2.322	2.315	**300**
patient **1**	1.241	1.260	1.250	**65**

TYPICAL STANDARD CURVE



Test validity

The test run is valid if:

- the mean OD of the standard 1 is ≤ 0.7
- the mean OD of the standard 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

CeliAK EmA human IgG	U/ml	BI
negative	< 20	< 1
positive	≥ 20	≥ 1

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-endomysial IgG levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by CeliAK EmA human IgG. However, anti-endomysial IgG autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

Due to the lack of an international reference material the CeliAK EmA human IgG is calibrated in arbitrary units (U/ml).

Linearity

Dilutions of selected positive specimens in anti-endomysial IgG autoantibody free human serum are determined according to their expected theoretical values with CeliAK EmA human IgG.

Sensitivity

The analytical sensitivity of the CeliAK EmA human IgG is 3 U/ml.

Diagnostic specificity and sensitivity

ROC analysis has been performed for Anti-hu tTG IgG measuring sera from 44 patients suffering from celiac disease and 117 healthy blood donors.

Using a cut-off value of 20 U/ml a specificity of 86.3 % and a sensitivity of 95.2 % was determined.

Precision

Intraassay (n = 8)		Interassay (n = 4 x 8)	
mean (U/ml)	CV %	mean (U/ml)	CV %
198.1	5.96	214.9	6.10
61.5	4.73	63.8	7.35
29.5	2.36	32.6	6.33
15.8	3.49	14.8	7.89

INCUBATION SCHEME

CeliAK EmA human IgG (4045)

Dilute patients sample	10 µl serum + 1.0 ml sample diluent (C)

1	Bring all ready for use reagents to room temperature (18…25°C) before use.				
			calibrators	control	sera
2	Pipette	Calibrators (0 - 4) or Calibrator 1	100 µl		
		Positive Control (P)		100 µl	
		prediluted 1 + 100 patient sera			100 µl
3	Incubate		60 minutes at room temperature		
4	Wash		Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature		
7	Wash		Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.