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Anti-Gliadin IgA

ORG 534A

96 Tests

**Immunoassay for the
quantitative determination of
anti-Gliadin Antibodies of
IgA class**

Instruction for use

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WARNINGS AND PRECAUTIONS

All reagents of this test kit are strictly intended for in vitro use only.

Please adhere strictly to the sequence of pipetting steps provided in this protocol. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

All reagents should be stored refrigerated at 2 - 8 °C in their original container.

Do not interchange kit components from different lots. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components beyond their expiration dates.

Allow all kit components and specimen to reach room temperature prior to use and mix well.

During handling of all kit reagents, controls and serum samples observe the existing legal regulations. The following precautions should be taken handling potentially infectious materials:

- do not eat, drink or smoke in areas where specimens or kit reagents are handled
- do not pipette by mouth
- wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

The test kit contains components of human origin which, when tested by FDA-licensed methods, were found negative for hepatitis B surface antigen and for HIV antibody. No known test can guarantee, however, that products derived from human blood will not be infectious. Handle, therefore, all reagents and human blood derivatives, like plasma or serum samples, as if capable of transmitting infection.

Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin wash thoroughly with water and soap.

The stop solution contains hydrochloric acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.

Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.

MATERIALS SUPPLIED

Package size	96 determ.
divisible microplate consisting of 12 modules of 8 wells each,.....	1
coated with Gliadin	
calibrators with IgA class Anti-Gliadin antibodies in a PBS/BSA	6 vials, 1.5 ml each
matrix containing: 0; 6; 12; 25; 50; 100 U/ml	
Anti-Gliadin controls in a PBS/BSA matrix (positive and negative),	2 vials, 1.5 ml each
for the respective concentrations see the enclosed package insert	
sample buffer, yellow, Concentrate	1 vial, 20 ml
enzyme conjugate solution, (light red) containing polyclonal	1 vial, 15 ml
rabbit anti-h-IgA-IgG; labelled with horseradish peroxidase	
TMB substrate solution	1 vial, 15 ml
stop solution (1 M hydrochloric acid)	1 vial, 15 ml
buffered wash solution, Concentrate	1 vial, 20 ml

CONTROLS

A set of two controls is provided with the kit.

TECHNICAL DATA

Sample material:	serum or plasma
Required sample volume:	10 µl of sample to be diluted 1:100 with sample buffer 100 µl prediluted sample per single determination
Total incubation time:	60 minutes at room temperature (20 - 28 °C)
Calibration range:	6 - 100 U/ml
Sensitivity:	0.5 U/ml
Storage:	refrigerated at 2 - 8 °C
Shelf life:	12 months after manufacturing or until the expiration date printed on the labels
Package size:	96 tests

PRINCIPLE OF THE PROCEDURE

Anti-Gliadin IgA is an indirect solid phase enzyme immunometric assay (ELISA). It is designed for the quantitative measurement of IgA class antibodies directed against Gliadin. Preparations of purified Gliadin from wheat are coated on the microplates.

The microplates can be divided into 12 modules of 8 wells each or can be used completely for 96 determinations. Each well can be separated from the module ("break-away").

The binding of present antibodies, formation of the sandwich complexes and enzymatic color reaction take place during three different reaction phases:

Phase 1:

Calibrators, controls and prediluted patient samples are pipetted into the wells of the microplate. Any present antibodies bind to the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

Phase 2:

An anti-human-IgA horseradish peroxidase conjugate solution is pipetted into the wells of the microplate to recognize IgA class antibodies bound to the immobilized antigens. After a 15 minutes incubation any excess enzyme conjugate, which is not specifically bound is washed away with wash buffer.

Phase 3:

A chromogenic substrate solution containing TMB (3,3',5,5'-Tetramethylbenzidine) is dispensed into the wells. During 15 minutes of incubation the color of the solutions change into blue. Color development is stopped by adding 1 M hydrochloric acid as stop solution. The solutions color change into yellow. The amount of color is directly proportional to the concentration of IgA antibodies present in the original sample.

To read the optical density a microplate reader with a 450 nm filter is required. Bi-chromatic measurement with a 600-690 nm reference is recommended.

CLINICAL RELEVANCE

The serological diagnosis of infant and adult forms of celiac disease is replacing more and more the invasive biopsy procedures. Celiac disease, also known as gluten sensitive enteropathy is primarily a disease of the infant organism. It is caused by a hypersensitivity reaction in response to gliadin, a protein being present in many cereals. This, non IgE mediated food allergy leads to massive malabsorption disturbances and is characterized by a complete atrophy of the villi and a hyperplasia of the crypts of the upper intestine.

Adhering to a gluten free diet, i.e. excluding all foods containing wheat, rye, oat and barley flour leads to a very rapid recovery of the mucosa and additionally the massive problems with malabsorptions and their accompanying clinical pictures disappear. Accordingly patients suffering from celiac disease must maintain a gluten free diet for the rest of their life.

Gliadins are proteins containing high amounts of the amino acids prolin and glutamine. This protein belongs to the nutritive tissue of the grain seeds of wheat, oat, barley and rye and is responsible for the baking properties of the flour.

Due to the possibilities of the highly specific and sensitive serological determination of IgA and IgG antibodies against gliadin the invasive procedures of biopsies can be given up. In the past several biopsies have been done with patients when celiac disease was suspected, after a period of a gluten-free diet and also after a specific gluten challenge

Gliadin antibodies titer correlate very well with the morphological appearance of the mucosa of the upper intestine. It has been well documented that gliadin antibodies level fall very quickly after a gluten free diet has begun and rise immediately after restoring gluten to the diet. Thus the serological test represents a reliable method to monitor patients, and in particular children and teenagers, for their adherence to the gluten-free diet.

NORMAL VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Gliadin tests:

	anti-Gliadin-Ab IgA [U/ml]
normal:	< 12
elevated:	≥ 12

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.

It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Gliadin.

SPECIFICITY

The microplate is coated with purified Gliadin from wheat. The test kit is specific only for antibodies against Gliadin.

CALIBRATION

Since no international reference preparation for Anti-Gliadin antibodies is available, the assay system is calibrated in relative arbitrary units.

REFERENCES

1. Chartrand LJ, Seidman EG.
Celiac disease is a lifelong disorder.
Clin.Invest.Med., Vol. 19, 357-361, 1996
2. Cornell HJ.
Coeliac disease: A review of the causative agents and their possible mechanisms of action.
Amino Acids, Vol. 10, 1-19, 1996
3. Cronin CC, Feighery A, Ferriss JB, Liddy C, Shanahan F, Feighery C.
High prevalence of celiac disease among patients with insulin-dependent (type I) diabetes mellitus.
Am.J Gastroenterol., Vol. 92, 2210-2212, 1997
4. Jokinen J, Peters U, Maki M, Miettinen A, Collin P.
Celiac sprue in patients with chronic oral mucosal symptoms.
J Clin.Gastroenterol., Vol. 26, 23-26, 1998
5. Taminiu JA.
Celiac disease.
Curr.Opin.Pediatr., Vol. 8, 483-486, 1996
6. Williams CN.
Celiac disease: past, present and future.
Can.J Gastroenterol., Vol. 11, 647-649, 1997

MATERIALS REQUIRED

Equipment

- Microplate reader capable for endpoint measurements at 450 nm
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl

Preparation of reagents

- distilled water
- graduated cylinder for 100 and 1000 ml
- plastic container for storage of the wash solution

Optional

- Multi-Chanel Dispenser
- or repeatable pipet for 100 µl
- data reduction software

SPECIMEN COLLECTION AND PREPARATION

For determination of Anti-Gliadin serum or plasma are the preferred sample matrices.

All serum and plasma samples are prediluted 1 : 100 with sample buffer. Therefore 10 µl of sample may be diluted with 1,000 µl of sample buffer.

The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation.

Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

PREPARATION AND STORAGE OF REAGENTS

All components of this test kit are supplied in a liquid format and ready to use, except the sample buffer and wash buffer. When stored refrigerated at 2 - 8 °C the components are stable for at least 30 days after opening or until the expiration date printed on the labels.

Remaining modules of the microplate should be stored refrigerated at 2 - 8 °C protected from moisture; store together with desiccant and carefully sealed in the plastic bag.

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of buffered wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

NOTES ON TECHNIQUE

Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

Pipetting and Sample Handling

Use a disposable-tip micropipette to dispense sera and plasma samples. Pipet directly to the bottom of the wells. To avoid carryover contamination change the tip between samples.

Patient samples expected to contain high concentrations should be additionally diluted with sample buffer before. Additional dilutions must be considered during calculation.

IMMUNOASSAY PROCEDURE

Do not interchange components of different lots.

All components should be at room temperature before use.

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 1000 µl of sample buffer in a polystyrene tube. Mix well. Calibrators and controls are ready to use and need not to be diluted.

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and prediluted patient samples in duplicates.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA - SF: standards A to F

P1, P2... patient sample 1, 2 ...

C1: positive control

C2: negative control

2. Pipet **100 µl of calibrators, controls and prediluted patient samples** into the wells.
3. Incubate for **30 minutes** at room temperature (20 - 28 °C).
4. Discard the contents of the microwells and wash **3 times with 300 µl of wash solution**.
5. Dispense **100 µl of enzyme conjugate** solution into each well.
6. Incubate for **15 minutes** at room temperature.
7. Discard the contents of the microwells and wash **3 times with 300 µl of wash solution**.
8. Dispense **100 µl of TMB substrate solution** into each well.
9. Incubate for **15 minutes** at room temperature protected from light.
10. Add **100 µl of stop solution** to each well of the modules and leave untouched for 5 minutes.
11. Read the optical density at **450 nm** and calculate the results. Bi-chromatic measurement with reference at 600-650 nm is recommended.

**The developed color is stable for at least 30 minutes.
Read optical densities during this time.**

CALCULATION OF RESULTS

For Anti-Gliadin IgA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

CALCULATION EXAMPLE

The figures below show typical results for Anti-Gliadin IgA. These data are in-tended for illustration only and should not be used to calculate results from another run.

No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl.Conc.	CV %
STA	A 1/B 1	0.006	0.009	0.008	0.0	0.1	0.0	0.0	28
STB	C 1/D 1	0.180	0.176	0.178	6.0	5.9	5.9	6.0	2
STC	E 1/F 1	0.367	0.374	0.371	12	12	12	12	1
STD	G 1/H 1	0.713	0.724	0.718	25	25	25	25	1
STE	A 2/B 2	1.245	1.200	1.223	52	49	50	50	3
STF	C 2/D 2	1.777	1.737	1.757	103	97	100	100	2

ASSAY CHARACTERISTICS

Sensitivity

The lower detection limits for Anti-Gliadin IgA were determined at 0.5 U/ml.

Parallelism

In dilution experiments sera with high IgA-antibody concentrations were diluted with sample buffer and assayed in the Anti-Gliadin IgA kit. The assay show linearity over the full measuring range.

INCUBATION SCHEME

- ① Pipet **100 µl** calibrator, control or diluted patient sample
→ Incubate for **30 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- ② Pipet **100 µl** enzyme conjugate
→ Incubate for **15 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- ③ Pipet **100 µl** substrate solution
→ Incubate for **15 minutes** at room temperature
- ④ Add **100 µl** stop solution
→ Leave untouched for **5 minutes**
→ Read at **450 nm**