Anti-Gliadin IgA

For In vitro diagnostic use only
Enzyme immunoassay for the quantitative determination
of antigliadin IgA antibodies in serum or plasma

SUMMARY

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

Gliadin the alcohol soluble fraction of gluten represents the causative agent of celiac disease that provokes an inflammatory process in the small intestine. Gliadin is a substrate of tissue transglutaminase and cross-linked into high molecular complexes triggering probably both cellular and humoral immune responses.

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.

Today diagnosis *of* celiac disease 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 anti-endomysium IgA by immunofluorescence has been considered as the main serological parameters for celiac disease so far.

Atlas Medical offers a complete range of serological markers for celiac disease including **Anti-Gliadin IgG, Anti-Gliadin IgA** and the novel **Anti-huTransG** and **Anti- hu tIG IgG,** ELISAs for the determination of IgA and IgG autoantibodies to tissue transglutaminase. All assays employ the same assay scheme and predilution maximizing laboratory efficiency.

PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by the antigen. Antibodies from the specimen bind coated antigen on the microwell surface. Unbound material is removed by washing procedure. Second antibodies directed towards human IgA , and labeled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of TMB mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of specific antibodies in the specimen.

KIT COMPONENTS

1. **microtiter strips:**12*8 well strips , coated with human Gliadin . the strips are contained in a sealed bag with dessicant.
Bring the strips to room temperature before use , to prevent any moisture formation inside the bag.
2. **Enzyme conjugate**: 1 vial of 11 ml.
Ready to use liquid reagent.
Stabilized proteic buffer solution containing anti-human IgA, conjugated with peroxidase (HRP)
3. **wash buffer**: 1 vial of 25 ml.
20 x concentrated solution to be diluted up to 500 ml with bidistilled water . it contains a phosphate buffer , a detergent and preservatives (0.1% Kathon GC).
4. **TMB**: 1 vial of 11 ml ready to use tetramethylbenzidine solution (TMB) with activators and stabilizers.
Avoid light exposure.
5.**stop solution** : 1 bottle containing ready to use 11 ml of 1 M hydrochloric and phosphoric acid.
Warning! The reagent is irritant:Xi R36/37/38: S(1/2) 26-45 Handle with care
6. standard 1:1 vial containing 1.1 ml .
Ready to use liquid reagent. Standard 0 IU/ml.
7. **gliadin IgA standards**: 1 vial containing 1.1 ml each ready to use liquid reagent containing Gliadin IgA at the following concentrations: S2: 25 IU/ml, S3: 50 IU/ml, S4: 100 IU/ml, and S5: 200 IU/ml.
8. **control** : 1 vial containing 1.1 ml.
Ready to use liquid reagent.
9. **sample diluent**: 1 bottle containing 50 ml .
ready to use liquid.
10. **package insert**

STORAGE AND STABILITY OF THE TEST

1. the kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. unused strips have to be placed in the bag containing the dessicant and firmly sealed before re-store at 2-8 °C.
3. the diluted wash buffer can be stored for one week at room temperature or 3 weeks at 2-8 °C.
4. all other liquid reagents are stable up to the expiry date stated on the labels, when stored at 2-8 °C and handled carefully to avoid any environment contamination.

SPECIMEN COLLECTION AND STORAGE

Serum or plasma (ACD or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.
Specimens can be stored up to 48 hours at 2-8 before testing. For a long storage, the specimens should be frozen at -20 or lower. Repeated freezing/thawing should be avoided.

MATERIALS REQUIRED BUT NOT PROVIDED

- semi automated pipettes of 10, 200, and 1000 µl.
- automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.
- photometric microstrips reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 – 630 nm.

- bidistilled water
- vortex mixer and absorbent paper
- chronometer.
- plate cover

PREPARATION BEFORE USE

WASH BUFFER: dilute 1:20 with bidistilled water and mix carefully before use.
For an immediate utilization , diluted washing solution can be stored at room temperature and is stable one week.
The other reagents are ready to use.

WASHING INSTRUCTIONS

A good washing procedure is essential to obtain correct and precise analytical results.
We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.
Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid folse positive reactions and remove high background.
Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.
In case of manual washing , we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.
In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5 % before being thrown away or autoclaved , as it must be considered as potentially infected.

ASSAY PROCEDURE

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. do not mix reagents of different lots.
3. we recommend to distribute the calibration set and the samples in duplicate.
4. distribution and incubation times must be the same for all wells in the same analysis.
5.avoid long interruptions between each step of the assay procedure.
6. it is suggested to eliminate the excess of washing by blotting it gently on an absorbent paper pad.
7. the color developed in the last incubation is stable for a maximum of one hour in the dark.
8. we recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm.
9. for the washing step , use only the wash buffer provided in the kit and follow carefully the indications reported in (washing instruction). It is recommended anyway to use a good quality microplate washer.
10. avoid the TMB to come in contact with oxidizing agents or metabolic surfaces; avoid intense light exposure during incubation.
11. **the blanking of the instrument is to be carried out in the standard 1 wells.**

ASSAY FLOWCHART

1. put the desidered number of microstrips into the frame; allocate two wells for each unknown sample and 12 wells for calibrators (standards) and control.
2. dilute all samples using sample diluent 101 fold (ex: 1ml + 10 µl sample). Do not dilute standards and control.
3. if suggested analyte concentration in the sample exceeds the highest calibrator, dilute this sample accordingly, using sample diluent.
4. dispense : 100 µl standards, 100 µl control, 100 µl sample
5. cover the strips with adhesive film.
6. incubate 30 minutes at 20-25 °C
7. peel out the adhesive film and aspirate the reaction solution from all the wells.
8. wash 3 times with 300 µl of diluted washing solution , carefully aspirating off the remaining liquid.
9. add 100 µl of enzyme conjugate
10. cover the strips with adhesive film
11. incubate 30 min at 20-25 °C
12. peel out the adhesive film and aspirate the reaction solution from all the wells.
13. wash 5 times with 300 µl of diluted washing solution , carefully aspirating off the remaining liquid.
14. add 100 µl TMB
15. cover the strips with adhesive film
16. incubate 15 minutes at room temperature, protected from light
17. add 100 µl stop solution
18. read the absorbance of each well, against standard 1 as blank, at 450 and 620-630 nm
19. apply point-by-point method for data reduction.

CALCULATIONS OF RESULTS

1. calculate the mean value of the OD 450 nm obtained for each duplicate.
2. subtract blank value (standard 1) to the mean OD 450 nm values of standards, control(s) and samples.
3. draw a standard curve by plotting the absorbances of the standards with the corresponding concentrations. A point-by-point method for data reduction is recommended. Alternatively , the calculation system of the microplate reader software can be used.
4. calculate the concentrations of control(s) and samples from the obtained standard curve.

QUALITY CONTROL
Control sample(s) should fit into the established range stated on the label(s) of the control(s).

EXPECTED VALUES
Based on data obtained by ATLAS , the following normal ranges are recommended (see below).
However , it is recommended that each laboratory establish it is own reference range.

Sex, age		Units:U/ml	
		Lower limit	Upper limit
<1year	1 month-5 years		35
1-6 years	6-9 years		45
6-12 years	10-11 years		40
> 12 years	12-17 years		25

Patients with all forms of interstitial lung diseases (hypersensitivity pneumonitis, idiopathic fibrosing alveolitis, fibrotic stages of sarcoidosis,etc.) show very high frequency of gliadin IgA (ca. 80% of cases) and/or gliadin IgG(ca. 70% of cases).
This phenomenon is not related to concomitant GI pathology and to the activity of lung diseases itself and is resistant to gluten-free diet. The clinical role of these findings is unclear.

CHARACTERISTICS OF THE ASSAY

Sensitivity
The analytical sensitivity of the Anti-Gliadin IgA is 5 IU/ml.
Correlation :
the present kit well correlates with similar ones in commerce.

SAFETY PRECAUTIONS
- **This** kit is for in vitro use only. Follow the working instructions carefully. 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 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.

REFERENCES

1. chartrand LJ, Seidman EG. Celiac disease is a lifelong disorder. Clin.invest.med., vol. 19, 357-361, 1996.
2. cornell HJ. Celiac disease: a review of the causative agents and their possible mechanics of action.
Amino acids, vol. 10, 1-19, 1996.
3. cronin CC, Feighery A, Ferriss JB, liddy C, Shanhan F, Feighery C. high prevalence of celiac disease among patients with insulin-dependent (typeI) 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. taminiau JA. Celiac disease. Curr. Opin . pediatr., vol. 8, 483-468, 1996.
6. Williams CN. Celiac disease: past, present and future.
Can. J Gastroenterol., vol. 11, 647-649, 1997.

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