EXPORT ONLY VERSION

Transglutaminase IgA ELISA Kit

REF **7044**

Quantitative ELISA test for the measurement of tissue transglutaminase IgA antibodies in human serum

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BIOMERICA

I. INTRODUCTION AND INTENDED USE

Biomerica's Transglutaminase IgA ELISA is a quantitative test for the presence of transglutaminase IgA antibodies in human serum.

Celiac disease (CD) or gluten-sensitive enteropathy is a chronic disease in which an intestinal mucosal lesion impairs nutrient absorption. While the exact etiology of celiac disease remains unknown, gliadin, the alcohol soluble fraction of wheat gluten, is clearly the toxic agent[(1)]. In a large majority of untreated celiac patients, high concentrations of antigliadin antibodies (AGA) are detected in saliva, intestinal secretions and in the blood stream[(2)]. These antibodies gradually disappear after gluten exclusion from the patient's diet.

It has long been postulated that CD is of autoimmune origin. Besides anti-gliadin antibodies, Chorzekski discovered in 1980 that virtually all untreated patients presented with anti-endomysial IgA antibodies. The detection of these antibodies became the most sensitive and earliest marker for CD. Unfortunately, the test, which relies on tissue sections from monkey endomysium and on indirect immunofluorescence, is rather expensive and difficult to apply to large numbers of samples. The exact nature of the "endomysial antigen" remained elusive until, in 1997, Dietrich et al demonstrated that it is in fact tissue transglutaminase [(8)]. This ubiquitous enzyme easily cross-links gliadin, resulting in gliadin-gliadin or gliadin-transglutaminase complexes. Dieterich's finding represents the first true autoantibody associated with CD, but the exact pathogenesis of the disease is far from elucidated.

In the laboratory, the determination of serum transglutaminase (TGA) IgA autoantibodies offers the ease-of-use, convenience and reproducibility of ELISA with clinical performances (sensitivity and specificity) of endomysial antibody testing. It has also the same limitations:

- CD patients with selective IgA deficiency will come out as false negatives.
- TGA antibodies represent an **early** marker for CD and cannot be used to monitor diet compliance in treated patients (which is best achieved with gliadin-IgA antibody testing)

While mucosal biopsy of the duodenal-jejunal junction (where lesions are to be found in both mild and severe forms) remains the first and essential step in the diagnosis of celiac disease[(3)], a characteristically flat mucosa is not specific. High circulating levels of TGA IgA antibodies will therefore confirm the suspicion of celiac disease before the results of a control biopsy after a few weeks on a gluten-free diet.

TGA antibody testing also represents a simple and inexpensive method to select more efficiently candidates for jejunal biopsy among children or adults clinically suspected of celiac disease.

Commonly, celiac disease begins in infancy after weaning and the introduction of cereals but the symptoms may disappear spontaneously in later childhood or adolescence, despite continued malabsorption; even these silent forms are a potential cause of delayed puberty, growth retardation or even dwarfism.

After this clinical remission, the classic symptoms of adult celiac disease usually appear during the third to sixth decade, but the disease may remain indefinitely undiagnosed. Mild or asymptomatic forms can be at the origin of unexplained anemia, hyposplenism or osteoporosis[(2, 5)].

While indiscriminate screening of the overall population is unlikely to be cost-effective, early detection of TGA antibodies in high risk populations would contribute to the prevention of consequences of chronic malabsorption. Individuals at risk include:

- short-stature children,
- unexplained anemia,
- unexplained hypocalcemia or osteo-malacia,
- delayed puberty,
- insulin-dependent diabetes,
- first-degree relatives of patients with CD,
- autoimmune thyroiditis,
- connectivitis,

II. PRINCIPLE OF THE TEST

Transglutaminase IgA is an enzyme immunoassay: polystyrene wells are coated with activated tissue transglutaminase (human, recombinant). Serum to be tested is incubated in the well. Antitransglutaminase antibodies, if present, are bound to the transglutaminase coated solid phase.

After washing, rabbit anti-human IgA conjugated with horseradish peroxidase is added. At the end of a second incubation, unbound conjugate is removed by washing. When enzyme substrate is added, a blue color develops if anti-transglutaminase antibodies are present in the well. 1N sulfuric acid is added to stop the enzyme reaction and the absorbance of controls or samples are measured using a plate reader with wavelength set at 450 nm.

III. WARNING AND PRECAUTIONS

All reagents provided with the kit are for *in vitro* diagnostic use only.

1. **Potential Biohazardous Material**

The Calibrators and Control contain a small amount of human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious.

2. **Stopping Solution**

Stopping Solution consists of 1N H_2SO_4. This is a strong acid and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

Precautions

- For *in vitro* diagnostic use.
- Do not mix reagents from different master lots.
- Do not use kit components beyond the expiration date.
- Reagents, specially the Chromogenic Substrate, should not be exposed to **strong light** during storage or incubation.
- Do not pipette by mouth.
- When opening and removing aliquots from the primary vials, avoid microbial or cross contamination of reagents.

- Comply with the number of wash cycles.
- Proceed though the different steps of the test protocol without interruption and comply with the prescribed incubation times.
- Check that the Chromogenic Substrate is colorless before addition.
- Avoid contact of sulfuric acid (Stop Solution) with skin or eyes. If contact occurs, immediately flush area with water.
- Careful pipetting technique is necessary for reproducible and accurate results.

IV. REAGENTS AND MATERIALS

Materials Supplied:

1. **PLA TGA IgA** = TGA IgA Microwell Strips ..... 12 x 8 strips
2. **DIL SPE** = Sample Diluent (1X) ..... 1 x 85.0 ml
3. **BUF WASH 30X** = Wash Buffer (conc.) ..... 1 x 13.0 ml
4. **CAL TGA IgA** = TGA IgA Calibrators (human serum) ..... 5 x 1.0 ml
5. **CTRL + IgA** = TGA IgA Positive Ctrl (human serum) ..... 1 x 1.0 ml
6. **CONJ ENZ IgA-HRP**= TGA IgA Enzyme Conjugate ..... 1 x 11.0 ml
7. **RGT B TMB SUBS** = Substrate Solution (TMB) ..... 1 x 20.0 ml
8. **SOLN STOPPING** = Stopping Solution (1N H_2SO_4) ..... 1 x 20.0 ml

V. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water.
- Micropipettes, 20 µl, 100 µl and 500 µl.
- Automatic or semiautomatic multi-channel micropipettes, 100 µl.
- Single or multichannel dispenser, 300 µl.
- Graduated cylinder, 500 ml.
- Buffer bottle, 0.5 L.
- Sample test tubes, polystyrene, 12 x 75 mm.
- Microplate reader with 450 nm wave-length absorbance capability.

VI. SPECIMEN COLLECTION

This kit has been validated with serum and plasma (heparin or EDTA) samples. If specimens are to be stored, excessive hemolysis and the presence of large clots or microbial growth in the test specimen may interfere with the performance of the test. Freeze the sample at -20° C if it cannot be analyzed within 24 hours.

VII. REAGENT PREPARATION AND STORAGE

The following reagent preparation steps are required before running the test:

1. **Wash Buffer**
   Pour the contents of the TGA Wash Buffer bottle in a 500 ml graduated cylinder. Adjust to 400 ml with deionized water. Transfer the resulting solution to a suitable plastic bottle, label it "Celiac Wash" and store it at 2 - 8° C.

2. **Serum sample dilution**
   Dilute 1:25 each patient serum sample with the Diluent:
   - Add **20 µl** of serum to **500 µl** of Sample Diluent.

   Mix thoroughly prior to use.

3. **Antigen coated plate preparation**
   The plate consists of 12 breakable strips of 8 wells, each coated with transglutaminase. Each serum, control, or blank will occupy one of these wells. Calculate the number of wells to be used (see further). Remove the plate from the plastic bag. Remove the strips by inverting the plate and pressing gently the bottom of wells. Break one strip if necessary. Return the strips and wells not required for the test run to the package. Reseal the bag and store at 2 - 8° C.

All kit components should be stored at 2 - 8° C and are stable to the labeled expiration date. DO NOT FREEZE KIT COMPONENTS.

NOTE: NEVER use a sample dilution prepared for transglutaminase autoantibody determination for antigliadin IgG or IgA antibody determination.

VIII. ASSAY PROCEDURE

1. Place the kit at room temperature for 30 minutes.
2. Place an appropriate number of strips and wells in the secure grid.

Determination of TGA IgA (duplicate determinations)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bl	Bl	S2	S2								
B	0	0	S3	S3								
C	3	3	S4	S4								
D	6	6	S5	S5								
E	25	25										
F	100	100										
G	C+	C+										
H	S1	S1										

Bl : Blank-well
0 : Calibrator 0 (undiluted)
3 : Calibrator 3 (undiluted)
6 : Calibrator 6 (undiluted)
25 : Calibrator 25 (undiluted)
100 : Calibrator 100 (undiluted)
C+ : Positive Control (undiluted)
S1 – S5 : Serum samples diluted 1:25

3. **Sample and control dispensing.**
   - Add 100 µl of Calibrators 0-100 into appropriate wells.
   - Add 100 µl of Positive Control.
   - Add 100 µl of each 1:25 sample dilution.

   Each well contains 100 µl of sample, control, or calibrator except wells 1A and 2A which serve as blanks
4. Cover and incubate the wells at room temperature (20 - 25° C) for **30 minutes**.
5. Discard the contents of the wells by gentle inversion and wash each wells by adding **300 µl Wash Buffer**. Repeat **two times**. Invert the strips over a paper towel and blot after each wash. Avoid air bubbles in wells. Finally, invert strips over a paper towel and blot dry. If you use an automated washer, check the manufacturer's instructions for a three cycle wash procedure with 300 µl wash volume.
6. Add 100 µl of the **Conjugate** to all wells, including blank wells. Cover wells and incubate at room temperature for **30 minutes**.
7. Repeat step 5.
8. Set a timer for 30 minutes, add **100 µl** of **Chromogenic Substrate** to the first well as you start the timer, and continue adding substrate in a steady rhythm through to the last well.
9. Allow the Substrate to react at room temperature (20-25 °C) for **30 minutes**. A blue color develops.
10. At 30 minutes, add **100 µl** of **Stop Solution** to each well including blank wells, in the same rhythm and the same order followed in step 8 above. When all the Stop Solution has been dispensed, mix the Stop Solution with Substrate by gently tapping the strips. A color change from blue to yellow is observed.
11. Blank the microtiterplate reader using wells 1A and 2A and read the absorbance of each well at 450 nm.

Summary of Assay Procedure

TGA IgA

Dilute samples :
20 µl serum + 500 µl Diluent
⇓
Pipette 100 µl **undiluted** calibrators/
100 µl **undiluted** control/
100 µl **diluted** sample(s)
⇓
Incubate 30 min.
⇓
Wash 3 times (3 x 300 µl)
⇓
Add IgA conjugate: 100 µl
⇓
Incubate 30 min. (RT)
⇓
Wash 3 times (3 x 300 µl)
⇓
Add substrate: 100 µl
⇓
Incubate 30 min. (RT)
⇓
Add stop solution: 100 µl
⇓
Read at **450 nm**

IX. CALCULATION OF DATA

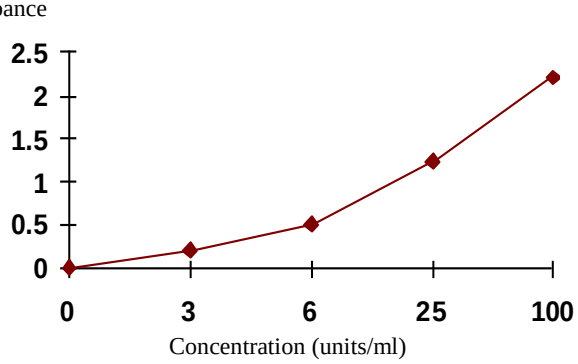
Manual Method

- Determine the mean absorbance for each pair of wells (calibrators, positive control, and samples).
- Plot a curve of absorbance on a semi-log or linear graph paper. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation.
- Read off sample concentrations directly from the curve. **Do not use any correction for dilution factor.**
- Any sample reading greater than the Calibrator 100 U/ml should be further diluted with the Sample Diluent and reassayed.

Automated Method

- Use an automated regression program to reduce the data, acceptable results will be obtained by using (1) Quadratic, (2) Cubic Spline or (3) Point to Point.
- Interpolate the patient values from the calibration curve**.**
- Any sample reading greater than the Calibrator 100 U/ml should be further diluted with the Sample Diluent and reassayed.

Example:



X. EXPECTED VALUES

- Samples with serum concentrations above **12** U/ml are considered positive.
- Samples with serum concentrations between **8** and **12** U/ml are considered equivocal.
- Samples with serum concentrations below **8** U/ml are considered negative.

XI. QUALITY CONTROL

If the absorbance of the Calibrator 0 U/ml is **≥ 0.250** at 450 nm, it may indicate a procedural problem and the test should be repeated.

Positive Control >12 U/mL

XII. LIMITATIONS

- The association between dermatitis herpetiformis and gluten-sensitive enteropathy is so strong that it has been suggested that both diseases have the same etiology; in these patients, TGA determination is useful to detect an asymptomatic celiac disease and to estimate the severity of the gastrointestinal involvement.

XIII. PERFORMANCE

Sensitivity

Minimum detectable dose : 3.5 U/ml

Precision

r = 12	Sample 1	Sample 2	Sample 3
Mean concentration (U/ml)	7.0	12	34
Standard deviation (U/ml)	0.6	0.7	1.7
Coefficient of variation (%)	8.5	5.8	5.0

Reproductibility

n = 8	Sample 1	Sample 2	Sample 3
Mean concentration (U/ml)	7.0	13	32
Standard deviation (U/ml)	1.1	1.2	2.1
Coefficient of variation (%)	15.7	9.2	6.5

XIV. CLINICAL DATA

Serum anti-transglutaminase in celiac disease and other gastrointestinal disorders.

Clinical status	n	No. of positive cases (%) for transglutaminase abs	No. of positive cases (%) for endomysial abs
Celiac disease - untreated	45	45 (100)	45 (100)
Celiac disease - treated	62	12(19.3)	13 (20.9)
Dermatitis herpetiformis - untreated	7	7 (100)	7 (100)
Dermatitis herpetiformis - dapsone treated	3	1 (33)	1 (33)
Other gastrointestinal disorders - adults	20	0	0
Other gastrointestinal disorders - children	35	0	0
Controls	120	3 (2.5)	4 (3.3)

XV. LITERATURE

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XVI. SYMBOLS

	Storage Temperature
	Lot Code
	Expiration
	Manufacturer
	Authorized Representative
	Caution, see instructions
	For in vitro diagnostic use
REF	Catalog No.

XVII. ORDERING INFORMATION

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IVD

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