



EL-tTG™ IgA/IgG

**An enzyme immunoassay
for the detection and measurement
of anti-tissue transglutaminase antibodies
of IgA and IgG isotype**

For professional use only

Instruction Manual

Catalog No.:

104-117

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INTRODUCTION

Name: EL-tTG™ IgA/IgG



Intended use:

The **TheraTest EL-tTG™ IgA/IgG** and **TheraTest EL-GLIA™ IgA/IgG Kits** are enzyme-linked immunosorbent assay (ELISA) test systems for the semi-quantitative measurement of IgA and IgG anti-tissue transglutaminase (tTG) and anti-gliadin antibodies in human serum. Detection and semi-quantitation of these antibodies is intended to aid the diagnosis of patients with gluten sensitive enteropathies: celiac disease and dermatitis herpetiformis, in conjunction with other clinical findings and laboratory tests.

Summary and Explanation

Gluten-sensitive enteropathy (GSE) is caused by intolerance to gluten, the protein of wheat, rye, barley and oats. GSE is characterized by chronic inflammation and destruction of the intestinal mucosa and flattening of the epithelium (“villous atrophy”), resulting in malabsorption (1). The classical form of GSE is celiac disease (CD), however, GSE is also present in patients with dermatitis herpetiformis (Duhring’s disease) (2, 3). The occurrence of CD has a bimodal distribution, manifesting both in young children and in adults in the fourth or fifth decades. Typical symptoms include diarrhea, abdominal pain, weight loss, anemia, fatigue and failure to thrive in children. However, CD disease can be silent, and most adult patients have minimal or atypical symptoms, or are completely asymptomatic (4, 5). The consequences of malabsorption due to undiagnosed CD are a source of significant morbidity. Atypical signs and symptoms include anemia (iron and folate deficiency), hypertransaminasemia, osteoporosis, peripheral neuropathy, mood disorders and reproductive failure (6-9). The risk of intestinal lymphoma is increased (10). Strict gluten-free diet resolves the symptoms and prevents late consequences.

GSE is a frequent, but underdiagnosed disease. Recent papers utilizing autoantibody determinations for screening for unrecognized CD have reported a prevalence of 1:53-1:300 (11, 12). There is a strong genetic predisposition for CD, which explains the familial occurrence of the disease (13). CD is 2-5 times more frequent in patients with other autoimmune diseases, especially in those with type 1 diabetes mellitus, thyroid diseases, rheumatoid arthritis and Sjögren’s syndrome (14-16). GSE is almost always present in patients with dermatitis herpetiformis (2, 3), and its prevalence is as high as 8-10% in IgA deficient subjects (17).

The diagnosis is based on the revised criteria of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) (18). These include a positive gut biopsy, showing histological evidence of CD, plus the demonstration of the presence of at least two of the following antibodies: endomysial antibodies (EMA), anti-gliadin antibodies (AGA) and anti-reticulin antibodies (ARA). The identification of tissue transglutaminase enzyme as the target antigen for EMA (19) made it possible to develop tTG-based solid phase immunoassays. Because of its high sensitivity and specificity, the anti-tTG test is recommended as a first-line diagnostic test and as a tool for population screening (20).

The TheraTest EL-tTG™ IgA/IgG test system detects both IgA and IgG class anti-tTG antibodies. The diagnostic sensitivity of IgA antibodies is 95-100%, whereas IgG antibodies are

not always present. However, patients with IgA deficiency produce only IgG antibodies, usually in high amount (17). In these patients the IgG anti-tTG antibodies have similar diagnostic value to IgA antibodies in the IgA sufficient population. Both IgA and IgG anti-transglutaminase antibodies are 95-100% specific for GSE. The antibody titer rapidly declines in patients adhering to a gluten-free diet, but remains high in those with dietary non-compliance (21). Regular determination of the antibody level is recommended as part of the patients' follow-up (21, 22).

Method description

The TheraTest EL-tTG™ IgA/IgG Test System is a solid phase enzyme immunoassay in a 96-well plate format for the measurement of IgA and IgG antibodies against tissue transglutaminase. Wells are coated with human recombinant tissue transglutaminase, and incubated with Specimens, Calibrators, Positive and Negative Controls. During the incubation, anti-tTG antibodies present in the test sample are bound to the solid phase antigen. The wells are subsequently washed, and isotype-specific horseradish-peroxidase labeled anti-human immunoglobulin antibody (enzyme conjugate) is added. After incubating the wells with the enzyme conjugate, unbound labeled antibody is removed by washing. A chromogenic substrate solution is then added to the wells, and the presence of antibodies to tissue transglutaminase is detected by a color change produced by the conversion of the substrate by the enzyme. The reaction is stopped, and the intensity of the color, which is proportional to the amount of the bound antibody, is read by an ELISA reader. The absorbance value in the blank well (incubated with Specimen Diluent) is subtracted from the values obtained with Specimens, Calibrators, and Controls.

WARNINGS AND PRECAUTIONS



Reagents Containing Human Source Material



Controls and Calibrators contain human serum. Treat as potentially infectious. The materials used to prepare the Calibrators and Controls were derived from human blood. When tested by FDA-cleared methods for the presence of antibody to HIV (Human Immunodeficiency Virus) and Hepatitis C Virus and for Hepatitis B Surface Antigen (HbsAg), the materials were nonreactive. While these methods are highly accurate, no test method can offer complete assurance that HIV, hepatitis virus or other infectious agents are absent. Therefore these materials and all patient specimens should be handled as though capable of transmitting infectious diseases. Human material should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 4th edition, 1999. HHS Publication (NIH and CDC). Web site: <http://bmbbl.od.nih.gov/>

Stop Reagent (2 mol/L Phosphoric Acid)

Corrosive! May cause burns upon contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amount of water for at least 15 minutes.

Hazardous Substance Risk & Safety Phrases

R34 - Causes burns.

S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection

S45 - In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Chromogen

Irritant! This product contains 3,3',5,5'-tetramethylbenzidine (TMB) ($\leq 0.05\%$), a chromogenic indicator of horseradish peroxidase activity. It has shown neither mutagenic nor carcinogenic effects in laboratory experiments (28).

Hazardous Substance Risk & Safety Phrases:

R36/37/38 – Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.

S24/25 – Avoid contact with skin or eyes.

S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36 – Wear suitable protective clothing.

S51 – Use only in well-ventilated areas.

Reagents Containing Sodium Azide

Calibrators and Controls contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush drain with large quantities of water to prevent azide build-up.

Hazardous Substance Risk & Safety Phrases:

R22 - Harmful if swallowed.

R36/37/38 - Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.

S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 - After contact with skin, wash immediately with plenty of water.

S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection

S46 - If swallowed, seek medical advice immediately and show this container label.

General Precautions and Information

1. Do not pipette by mouth.
2. Do not eat, drink, or smoke in designated work areas.
3. Wash hands thoroughly after using specimens and kit reagents.
4. Do not use test components beyond the expiration date.
5. Work in a well ventilated area when using kit components.
6. Avoid exposing reagents to excessive heat or light during storage.
7. Chromogen contains an organic solvent that irritates eyes and mucous membranes.
8. Do not allow the Chromogen to come in contact with metal or oxidizing agents.
9. Use disposable glassware and plastic ware or wash all material thoroughly according to standard laboratory practice.
10. tTG/Gliadin 10X Wash Buffer, Chromogen and Stop reagent are interchangeable among EL-Glia™ and EL-tTG™ kits from different lots. All other reagents are test and lot specific and therefore are not interchangeable.
11. Avoid microbial contamination of the reagents.
12. Dispose of containers and unused kit reagents in accordance with local regulatory requirements.

STORAGE AND HANDLING

1. Store all reagents at 2° - 8°C when received. Avoid freezing reagents.
2. All reagents must be brought to room temperature (18° - 25°C) for 30 minutes prior to use.
3. Avoid direct sunlight.

IMPORTANT: When stored at 2° - 8°C, the tTG/Gliadin 10X Wash Buffer may form crystals. The crystals must be dissolved prior to dilution of the 10X concentrate when only a portion of the concentrate is being diluted. If all of the bottled contents are transferred at once to a 1-L graduated cylinder, be sure to rinse the bottle multiple times with water to dissolve and transfer any crystallized salts. When stored at 2° - 8°, the tTG/Gliadin 10X Wash Buffer is stable until kit expiration, the 1X Wash Buffer is stable for 8 weeks.

SPECIMEN REQUIREMENTS

Collection and Storage of Serum

A whole blood specimen should be obtained using accepted medical techniques to avoid hemolysis. The blood should be clotted and the serum separated by centrifugation within 24 h of collection. Grossly hemolyzed, lipemic or icteric serum is not acceptable, since it may affect the results of the test. Serum should be stored at 2° - 8 °C for up to 14 days prior to testing. If testing cannot be completed within 14 days of collection, the separated serum must be stored at -20°C. Do not use serum that has been thawed more than once or which has been heat inactivated. Serum samples have been tested for stability at room temperature and are stable with no apparent loss of antibody activity for seven days. The performance of plasma samples has not been evaluated; therefore plasma should not be used in the test.

PROCEDURE

Before starting the assay, read the product insert carefully. Instructions should be followed exactly as they appear in this kit insert to ensure valid results.

Materials Provided

1. **Antigen Coated Wells in 96-well plate format:** For single use only! All wells are coated with human recombinant tissue transglutaminase. Wells are printed with the name of the antigen. The unused wells and the frame may be stored and used at a later date. They are returned to their desiccant-containing pouch, which is sealed and stored dry at 2° - 8°C.
2. **tTG/Gliadin 10X Wash Buffer, 100 mL:** 10X concentrated buffer
3. **IgA Calibrator, 1.5 mL*:** Contains human serum with IgA antibodies to tTG and preservative in stabilizing buffer. See Data Sheet for performance characteristics.
4. **IgG Calibrator, 1.5 mL*:** Contains human serum with IgG antibodies to tTG and preservative in stabilizing buffer. See Data Sheet for performance characteristics.
5. **IgA Positive Control, 0.35 mL*:** Human serum containing IgA antibodies to tTG and preservative in stabilizing buffer. See Data Sheet for performance characteristics.
6. **IgG Positive Control, 0.35 mL*:** Human serum containing IgG antibodies to tTG and preservative in stabilizing buffer. See Data Sheet for performance characteristics.
7. **Negative Control, 0.35 mL*:** Human serum without IgA and IgG antibodies to tTG and with

preservative in stabilizing buffer. See Data Sheet for performance characteristics.

8. **tTG/Gliadin anti-IgA conjugate, 15 mL:** Goat anti-human IgA (α -chain specific) conjugated with horseradish peroxidase, with preservative and yellow dye.
9. **tTG/Gliadin anti-IgG conjugate, 15 mL:** Goat anti-human IgG (Fc γ specific) conjugated with horseradish peroxidase, with preservative and green dye.
10. **Chromogen, 27 mL:** 3,3',5,5' tetramethylbenzidine (TMB) in buffer with hydrogen peroxide.
11. **Stop Reagent, 27 mL:** 2 mol/L phosphoric acid.
12. **Resealable pouch.**

*Reagents containing sodium azide

Materials required but not provided

1. In addition to the reagents supplied, the following materials are required:
2. Calibrated precision micropipettes with disposable plastic tips that deliver 10 μ L, 100 μ L and 1 mL.
3. Calibrated adjustable multichannel pipettes (8- or 12-channel).
4. Disposable pipette tips.
5. Microtubes, polypropylene (dilution tubes or cluster tubes) with a rack of 96-well format.
6. Timer.
7. Pipettes (1 mL, 5 mL, and 10 mL).
8. Pipette reagent reservoirs (to accommodate multichannel pipettes).
9. Deionized or distilled water.
10. Single (450 nm) or dual (450 nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA plate reader) for 96-well microtiter plates.
11. Clean wash bottle and automated plate washer (optional).
12. Absorbent paper towels for blotting plates after well washes.

Reagent preparation:

1. Coated Wells

Testing is performed for IgA and IgG antibodies at the same time; suggested plate arrangements of wells are shown on the Data Sheet. The entire strip (or strips) may be employed, or individual wells may be used as desired.

2. Wash Solution

The tTG/Gliadin 10X Wash Buffer must be diluted 1:10 with deionized or distilled water prior to use. Prepare 1X Wash Buffer by pouring the contents of the tTG/Gliadin 10X Wash Buffer into a clean one liter volumetric container. Rinse the bottle with deionized or distilled water to remove residual buffer and redissolve any existing crystals. Add the rinse to the one liter container. Add deionized or distilled water until a total volume of 1.0 L is reached; mix thoroughly. Diluted Wash Buffer is stable for 8 weeks at 2° - 8 °C.

3. Specimens, Positive Control, Negative Control

The Specimens and Controls must be diluted 1:101 in tTG/Gliadin 1X Wash Buffer prior to being tested. Use high accuracy pipettes. For example, pipette 10 μ L of serum into 1 mL of 1X Wash Buffer. Discard any unused diluted Specimens and Controls after the test procedure is completed.

4. Calibrators

The Calibrators are provided prediluted. They are ready to use.

Assay Procedure

1. Allow all reagents and patient sera to equilibrate to room temperature prior to use (18° to 25°C). Plates should equilibrate to room temperature in their sealed foil pouch to prevent condensation.
2. Mark the position of the samples (i.e., Diluent Blank, Calibrator, Positive Control, Negative Control, and Specimens) on a worksheet, and arrange dilution tubes accordingly in a rack. A suggested plate arrangement is shown on the Data Sheet. IgA and IgG antibodies are measured at the same time on alternate strips.
3. Determine the number of 1 x 8-well strips needed. The remaining unused strips should be returned and resealed in the pouch with desiccant for later use.
4. Dispense 1 mL of tTG/Gliadin 1X Wash Buffer into each dilution tube.
5. Dilute all serum Specimens and Controls 1:101 (e.g. add 10 µL of serum to 1mL tTG/Gliadin 1X Wash Buffer) and mix well. Do not dilute Calibrators.
6. Pipette 100 µL of the Calibrators, diluted Samples and Controls into the appropriate wells. Set up one well in which only tTG/Gliadin 1X Wash Buffer is added; this well is to be used as a Diluent Blank. For best results pipette all materials within 5 minutes from the start of the assay. This step is facilitated by the use of multichannel pipettes.
7. Incubate the plate for 30 - 35 minutes at room temperature (18° - 25 °C).
8. Aspirate or decant the contents of the wells and wash the plate 3 times with 300 µL of tTG/Gliadin 1X Wash Buffer. An automated plate washer may be used for this step. Remove all residual liquid from the wells by inverting and blotting the plate on absorbent paper.
9. Immediately pipette 100 µL of IgG and IgA Enzyme Conjugates into the wells of alternate strips.
10. Incubate plate(s) for 30 - 35 minutes at room temperature (18° - 25 °C).
11. Aspirate or decant Enzyme Conjugates from all wells and wash the plate as in Step 8 above.
12. Immediately dispense 100 µL of Chromogen into each well. Incubate the plate(s) for 15(±1) min. at room temperature (18° - 25 °C). Blue color develops in the wells of the Calibrators and Postive Controls, as well as in positive test samples should they exist.
13. Pipette 100 µL of Stop Reagent into each well and mix by gently tapping the side of the plate. The blue color changes to yellow.
14. Determine the absorbance of each well at 450 nm using a single or dual wavelength spectrophotometer (ELISA reader). Absorbance values should be read within 30 minutes of completing the assay. For a dual wavelength spectrophotometer, set test wavelength at 450 nm with the reference between 620 and 690 nm.

Procedural Notes

1. Storage

Place unused strips in the open metallized pouch (with desiccant) for light protection and place this assembly into the provided resealable pouch and store at 2-8 °C.

2. Pipetting

To avoid cross-contamination and sample carryover, pipette the Calibrator, Positive Control,

Negative Control, and Specimens using separate pipette tips. A multi-channel pipette may be used to pipette the Enzyme Conjugates, Wash Solution, Chromogen and Stop Reagent.

3. Washing

Each column of wells may be washed using a multi-channel pipette. The wells may be aspirated using an appropriate vacuum apparatus, fitted with a Pasteur pipette, or their contents may be dumped into a disposal container. Alternatively, commercial semi-automated washing systems may be used. When using either washing technique, the plate should be inverted and blotted against absorbent paper after the last wash. Use reagent grade water only (CAP type 1 or USP grade) for preparing the tTG/Gliadin 1X Wash Buffer.

4. Measurement of Absorbance Values

Absorbance values should be measured within 30 minutes after completion of assay. If the absorbance value exceeds the detection limit of the instrument, an approximate value may be obtained by one of two methods:

- a) Dilute the end product (developed well) with deionized or distilled water to bring the absorbance value within the capacity of the reader (e.g., remove 100 μL of end product and add 100 μL of water). Multiply the measured value by the dilution factor. **LIMITATION:** Patient's antibody level may exceed the available antigenic sites of the well.
- b) Repeat the assay testing the specimen at 1:1010 or greater dilution (e.g. dilute the already 1:101 diluted specimen to 1:10 by adding 100 μL of diluted specimen to 900 μL of tTG/Gliadin 1X Wash Buffer). Multiply the measured value by the dilution factor.

QUALITY CONTROL

1. Diluent Blank

If the value of the diluent blank exceeds 0.150, the assay should be repeated. If a more detailed explanation is needed, please contact the manufacturer.

2. Positive and Negative Controls

Positive and Negative Controls must be run in each assay. The Positive and Negative Control values must fall within the ranges provided on the enclosed Data Sheet. If the values are not in agreement with the Data Sheet, the assay is not valid and the results should not be reported.

RESULTS

Calculation of Results

Most ELISA readers are computer compatible and data may be calculated with the help of computer programs. TheraTest Laboratories, Inc. can provide TERIS /mv software for computer-assisted calculations. Check periodically that the program chosen yields the same results as obtained by manual calculations.

1. Determination of absorbance values:

The specific absorbance (net absorbance) for each sample is calculated by subtracting the absorbance value of the Diluent Blank well from the absorbance value of the Specimen well. The same Blank well value is used for all test Specimens, Calibrator and Controls.

EXAMPLE:

Absorbance for Blank well = 0.050

Absorbance for Specimen well = 1.150

Net absorbance for the Specimen is $1.150 - 0.050 = 1.100$

Note: If the absorbance in the blank well is higher than in the Specimen well, the net absorbance should be considered zero.

2. Calculation of antibody activity

Antibody activity is calculated as follows:

$$\text{Conversion Factor} = \frac{\text{Unit value of the Calibrator}}{\text{Absorbance (OD) value of the Calibrator}}$$

Antibody Units in Specimen = Conversion Factor x Absorbance value of Specimen (U/mL)

3. Interpretation

The results should be considered normal and abnormal (positive) as follows:

Test	Normal	Equivocal	Abnormal
Anti-tTG IgA	≤ 20 U/mL	21-25 U/mL	> 25 U/mL
Anti-tTG IgG	≤ 20 U/mL	21-25 U/mL	> 25 U/mL

The reference range should be verified by each laboratory to reflect the characteristics of the population. When the results are equivocal it is recommended to report them as equivocal, and repeat the test at a later date on a different bleeding.

LIMITATIONS OF THE PROCEDURE

1. The Positive Control and the Calibrator for a specific antibody may contain other antibodies, i.e. they may not be monospecific.
2. The TheraTest EL-tTG™ IgA/IgG assay should not be performed on grossly hemolyzed, lipemic, icteric, or microbially contaminated samples.
3. This method has been tested using serum samples only. The performance using other types of specimens has not been determined.
4. Diagnosis should not be made solely on the basis of a positive test result. The results must be interpreted in conjunction with all clinical information available to the physician (i.e. history, physical exam and other diagnostic procedures).
5. This assay has not been evaluated on pediatric populations.

EXPECTED VALUES

The prevalence of anti-tTG antibodies has been reported by several studies, and the findings are summarized in **Table 1.** (23-26).

Table 1. *Reported sensitivity and specificity of IgA and IgG anti-tTG antibodies in celiac disease (23-26).*

	Sensitivity *	Specificity *
Anti-tTG IgA	96-100%	94-100%
Anti-tTG IgG	69-99%	94-95%

*Sensitivity and specificity values for untreated, IgA sufficient celiac disease patients.

IgA anti-tTG antibodies are sensitive and specific diagnostic markers for CD. IgG anti-tTG antibodies are not always present in IgA sufficient CD patients, but they are effective in identifying IgA-deficient CD subjects, because IgG anti-tTG antibodies are present in high titer in the absence of IgA anti-tTG antibodies. The level of anti-tTG antibodies also depends on the diet. The antibody level declines and may become negative in patients adhering to a gluten-free diet.

Simultaneous determination of IgA and IgG anti-gliadin antibodies (AGA) is recommended. The following algorithm can serve as a guide for the interpretation of antibody results (17, 20, 27):

Serological testing: IgA and IgG anti-tTG and IgA and IgG anti-gliadin

Result:	Probability of CD:
All negative:	very low
IgA anti-tTG positive (regardless of the status of other antibodies):	high*
IgA anti-gliadin positive without IgA anti-tTG	low
IgG anti-tTG and/or IgG anti-gliadin positive without IgA antibodies:	check IgA level in serum
- if serum IgA <0.05 g/l:	high*
- if serum IgA >0.1 g/l:	very low

*Celiac disease needs to be confirmed by gastroduodenoscopy and positive histology.

A total of 200 specimens from various groups of patients and controls were tested with the *TheraTest EL-tTG™ IgA/IgG* assay. The results are presented in **Table 2**.

Table 2. *Frequency of (A) anti-tTG IgA and (B) anti-tTG IgG antibodies measured by the TheraTest EL-tTG™ IgA/IgG assay in celiac disease patients and various groups of controls (n=200)*

A)

		Diagnosis		
		Celiac disease* (n=44)	Control group (n=100 blood bank donors, n=40 inflammatory bowel dis., n=16 thyroid disease)	Total (n=200)
EL-tTG™ IgA	Positive	42	3	45
	Equivocal	0	0	0
	Negative	2**	153	155
	Total	44	156	200

* The group includes patients on gluten-containing and gluten-restricted diet, as well.

** IgA deficient patients

Sensitivity: 95% (42/44)

Specificity: 98% (153/156)

B)

		Diagnosis		
		Celiac disease* (n=44)	Control group (n=100 blood bank donors, n=40 inflammatory bowel dis., n=16 thyroid disease)	Total (n=200)
EL-tTG™ IgG	Positive	21	3	24
	Equivocal	4	1	5
	Negative	19	152	171
	Total	44	156	200

* The group includes patients on gluten-containing and gluten-restricted diet, as well.

Sensitivity: 48% (21/44)

Specificity: 97% (152/156)

PERFORMANCE CHARACTERISTICS

Comparative studies

A total of 106 samples (42 from IgA sufficient CD patients, 14 from IgA deficient CD patients and 50 from healthy donors) were tested by the *TheraTest EL-tTG™ IgA/IgG* assay and another commercially available anti-tTG IgA and IgG ELISA. The results are presented in **Tables 3 & 4**.

Table 3. *TheraTest EL-tTG™ IgA versus another anti-human tTG IgA ELISA (n=106)*

		Other ELISA		
		Positive	Negative	Total
EL-tTG™ IgA	Positive	40	2	42
	Negative	0	64	64
	Total	40	66	106

Positive agreement: 40/40; 100% (95% CI: 91%-100%)
 Negative agreement: 64/66; 97% (95% CI: 89%-100%)
 Total agreement: 104/106; 98% (95% CI: 93%-100%)

Table 4. *TheraTest EL-tTG™ IgG versus another anti-human tTG IgG ELISA (n=106)*

		Other ELISA			
		Positive	Equivocal*	Negative	Total
EL-tTG™ IgG	Positive	27	(4)	3	30
	Equivocal*	0	(1)	(3)	(4)
	Negative	5	(1)	62	67
	Total	32	(6)	65	97

*Equivocals were excluded from the calculation.

Positive agreement: 27/32; 84% (95% CI: 67%-95%)
 Negative agreement: 62/65; 95% (95% CI: 87%-99%)
 Total agreement: 89/97; 92% (95% CI: 84%-96%)

Moreover, a total of 44 samples from CD patients were tested with the *TheraTest EL-tTG™ IgA* assay and with an anti-endomysium antibody assay. The anti-tTG IgA antibody results were in 100% concordance with the results of the anti-endomysium antibody test.

Cross-reactivity, i.e. specificity based on disease controls

Testing was performed with 56 sera consisting of specimens from of 40 inflammatory bowel disease (IBD) and 16 thyroid disease patients. The positive samples were: one IBD with anti-tTG IgA, one IBD with anti-tTG IgG, two IBD with anti-gliadin IgA, one IBD with anti-gliadin IgG and one thyroid disease with anti-gliadin IgG.

Precision

Specimens with three levels of reactivity (high, moderate and low positive) were selected for each assay. The specimens were tested 20 times within the same respective assay (intra-assay variation) and 20 different times in one or two runs per day (inter-assay variation). The results are presented in **Tables 5** and **6**.

Table 5. *Intra- and inter-assay variation in the Theratest EL-tTG™ IgA assay*

A)	
Anti-tTG IgA (U/mL)	Intra-assay CV%
187	8.2
96	2.8
26	10.9

B)		
Anti-tTG IgA (U/mL)		Inter-assay CV%
193		3.9
46		6.1
30		10.1

Table 6. *Intra- and inter-assay variation in the Theratest EL-tTG™ IgG assay*

A)		
Anti-tTG IgG (U/mL)		Intra-assay CV%
83		5.0
68		2.9
29		5.3

B)		
Anti-tTG IgG (U/mL)		Inter-assay CV%
83		7.8
68		9.3
27		10.7

TROUBLESHOOTING

Problem	Possible Causes	Solution
Control values out of range.	<ol style="list-style-type: none"> 1. Incorrect temperature, timing or pipetting; reagents not mixed. 2. Cross-contamination of controls. 3. Improper dilution. 4. Optical pathway not clean. 5. Wavelength of filter incorrect. 6. Specimen diluent blank >0.150 	<ol style="list-style-type: none"> 1. Check that temperature was correct. Check that time was correct. See “Poor Precision” (below) No. 2-4. Repeat test. 2. Pipette carefully. 3. Repeat test. 4. Check for moisture or dirt. Wipe bottom and reread. 5. Change filter to 450 ± 5 nm. 6. Check for contamination of Chromogen or conjugate solution.
All test results negative.	<ol style="list-style-type: none"> 1. One or more reagents not added, or added in wrong sequence. 2. Improper dilution of wash buffer. 3. Antigen coated plate inactive. 	<ol style="list-style-type: none"> 1. Recheck procedure. Check for unused solutions. Repeat test. 2. Repeat test. 3. Check for obvious moisture in unused wells. Rerun test with controls only for activity.
All test results yellow Scattered false positives	<ol style="list-style-type: none"> 1. Contaminated chromogen. 2. Contaminated buffers/reagents 3. tTG/Gliadin 1X Wash Buffer contaminated. 4. Improper dilution of serum. 5. Contaminated pipette 	<ol style="list-style-type: none"> 1. Check absorbance of unused chromogen. 2. Check all solutions for turbidity. 3. Use clean container for 1X Buffer. Check quality of water used to prepare buffer. 4. Repeat test. 5. Use felt-plugged tips for chromogen
Poor precision.	<ol style="list-style-type: none"> 1. Pipettor delivery CV greater than 5%. 2. Serum or reagents not mixed sufficiently; reagents not at room temperature prior to addition. 3. Reagent addition taking too long; inconsistency in timing intervals, air bubbles. 4. Air currents blowing over plate during incubations. 5. Optical pathway not clean. 6. Instrument not equilibrated before readings were taken. 7. Washing not consistent; trapped bubbles; liquid left in wells at end of wash cycle. 8. Improper pipetting. 	<ol style="list-style-type: none"> 1. Check calibration of pipettor. Use reproducible technique. 2. Mix all reagents gently but thoroughly and equilibrate to room temperature. 3. Develop consistent uniform technique and avoid splashing or use multi-channel device or autodispenser to decrease time. 4. Cover plate or place in chamber. 5. Wipe bottom of plate with soft tissue. Check instrument light source and detector for dirt. 6. Check instrument manual for warm up procedure. 7. Use only acceptable washing devices. Lengthen timing delay on washing devices. Check that all wells are filled. 8. Avoid air bubbles in pipette tips.

REFERENCES

1. Schuppan D, Dennis MD, Kelly CP. Celiac disease: epidemiology, pathogenesis, diagnosis, and nutritional management. *Nutr Clin Care*. 2005;8:54-69.
2. Reunala T. Dermatitis herpetiformis: coeliac disease of the skin. *Ann Med*. 1998;30:416-8.
3. Kumar V, Jarzabek-Chorzelska M, Sulej J, Rajadhyaksha M, Jablonska S. Tissue transglutaminase and endomysial antibodies-diagnostic markers of gluten-sensitive enteropathy in dermatitis herpetiformis. *Clin Immunol*. 2001;98:378-82.
4. Nelsen DA Jr. Gluten-sensitive enteropathy (celiac disease): more common than you think. *Am Fam Physician*. 2002 Dec 15;66:2259-66.
5. Tikkakoski S, Savilahti E, Kolho KL. Undiagnosed coeliac disease and nutritional deficiencies in adults screened in primary health care. *Scand J Gastroenterol*. 2007;42:60-5
6. Stenson WF, Newberry R, Lorenz R, Baldus C, Civitelli R. Increased prevalence of celiac disease and need for routine screening among patients with osteoporosis. *Arch Intern Med*. 2005;165:393-9.
7. Hadjivassiliou M, Grunewald RA, Kandler RH, Chattopadhyay AK, Jarratt JA, Sanders DS, Sharrack B, Wharton SB, Davies-Jones GA. Neuropathy associated with gluten sensitivity. *Neurol Neurosurg Psychiatry*. 2006;77:1262-6.
8. Stazi AV, Mantovani A. A risk factor for female fertility and pregnancy: celiac disease. *Gynecol Endocrinol*. 2000;14:454-63.
9. Halfdanarson TR, Litzow MR, Murray JA. Hematological manifestations of celiac disease. *Blood*. 2007;109:412-21.
10. Smedby KE, Akerman M, Hildebrand H, Glimelius B, Ekbohm A, Askling J. Malignant lymphomas in coeliac disease: evidence of increased risks for lymphoma types other than enteropathy-type T cell lymphoma. *Gut*. 2005;54:54-9.
11. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med*. 2003;163:286-92.
12. Rostami K, Mulder CJ, Werre JM, van Beukelen FR, Kerchhaert J, Crusius JB, Pena AS, Willekens FL, Meijer JW. High prevalence of celiac disease in apparently healthy blood donors suggests a high prevalence of undiagnosed celiac disease in the Dutch population. *Scand J Gastroenterol*. 1999;34:276-9.
13. Spurkland A, Sollid LM, Ronningen KS, Bosnes V, Ek J, Vartdal F, Thorsby E. Susceptibility to develop celiac disease is primarily associated with HLA-DQ alleles. *Hum Immunol*. 1990;29:157-65.
14. Gillett PM, Gillett HR, Israel DM, Metzger DL, Stewart L, Chanoine JP, Freeman HJ. High prevalence of celiac disease in patients with type 1 diabetes detected by antibodies to endomysium and tissue transglutaminase. *Can J Gastroenterol*. 2001;15:297-301.
15. Luft LM, Barr SG, Martin LO, Chan EK, Fritsler MJ. Autoantibodies to tissue transglutaminase in Sjögren's syndrome and related rheumatic diseases. *J Rheumatol*. 2003;30:2613-9.
16. Cuoco L, Certo M, Jorizzo RA, De Vitis I, Tursi A, Papa A, De Marinis L, Fedeli P, Fedeli G, Gasbarrini G. Prevalence and early diagnosis of coeliac disease in autoimmune thyroid disorders. *Ital J Gastroenterol Hepatol*. 1999;31:283-7.
17. Korponay-Szabo IR, Dahlbom I, Laurila K, Koskinen S, Woolley N, Partanen J, Kovacs JB, Maki M, Hansson T. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. *Gut*. 2003;52:1567-71.
18. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK. Revised criteria for diagnosis of coeliac disease: Report of Working Group of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN). *Arch Dis Child* 1990;65:909-911.

19. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med.* 1997;3:797-801.
20. Gomez JC, Selvaggio G, Pizarro B, Viola MJ, La Motta G, Smecuol E, Castelletto R, Echeverria R, Vazquez H, Mazure R, Crivelli A, Sugai E, Maurino E, Bai JC. Value of a screening algorithm for celiac disease using tissue transglutaminase antibodies as first level in a population-based study. *Am J Gastroenterol.* 2002;97:2785-90.
21. Bazzigaluppi E, Roggero P, Parma B, Brambillasca MF, Meroni F, Mora S, Bosi E, Barera G. Antibodies to recombinant human tissue-transglutaminase in coeliac disease: diagnostic effectiveness and decline pattern after gluten-free diet. *Dig Liver Dis.* 2006;38:98-102.
22. Burgin-Wolff A, Dahlbom I, Hadziselimovic F, Petersson CJ. Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. *Scand J Gastroenterol.* 2002;37:685-91.
23. Lerner A, Kumar V, Iancu TC. Immunological diagnosis of childhood coeliac disease: comparison between antigliadin, antireticulin and antiendomysial antibodies. *Clin Exp Immunol.* 1994 Jan;95:78-82.
24. Basso D, Gallo N, Guariso G, Pittoni M, Piva MG, Plebani M. Role of anti-transglutaminase (anti-tTG), anti-gliadin, and anti-endomysium serum antibodies in diagnosing celiac disease: a comparison of four different commercial kits for anti-tTG determination. *J Clin Lab Anal.* 2001;15:112-5.
25. Wong RC, Wilson RJ, Steele RH, Radford-Smith G, Adelstein S. A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits. *J Clin Pathol.* 2002;55:488-94.
26. Reeves GE, Squance ML, Duggan AE, Murugasu RR, Wilson RJ, Wong RC, Gibson RA, Steele RH, Pollock WK. Diagnostic accuracy of coeliac serological tests: a prospective study. *Eur J Gastroenterol Hepatol.* 2006 May;18:493-501.
27. Viola L, Lugli L, Melegari A, Marotti E, Amarri S, Balli F. Antitransglutaminase enzyme-linked immune-adsorbed assay in coeliac disease diagnosis: evaluation of a diagnostic algorithm. *Pediatr Med Chir.* 2004;26:126-31.
28. Garner RC. Testing of some benzidine analogues for microsomal activation to bacterial mutagens. *Cancer Lett* 1975, 1:39-42.

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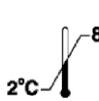
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EL-tTGTM IgA/IgG Abbreviated Test Procedure

- 1. Dilute Controls and Specimens 1:101 with tTG/Gliadin 1X Wash Buffer.**
- 2. Pipette 100 μ L of Calibrators, diluted Controls and Specimens into appropriate wells (see Data Sheet for configuration); add only specimen diluent (tTG/Gliadin 1X Wash Buffer) to one well (Diluent Blank).**
- 3. Incubate for 30-35 minutes at room temperature (18-25°C).**
- 4. Wash the wells three times with tTG/Gliadin 1X Wash Buffer.**
- 5. Add 100 μ L of IgA and IgG Enzyme Conjugate into appropriate wells.**
- 6. Incubate for 30-35 minutes at room temperature (18-25°C).**
- 7. Wash the wells three times with tTG/Gliadin 1X Wash Buffer.**
- 8. Add 100 μ L of Chromogen into each well.**
- 9. Incubate for 15 \pm 1 minutes at room temperature (18-25°C).**
- 10. Add 100 μ L of Stop Reagent into each well.**
- 11. Read the absorbance at 450 nm (reference wavelength 620-690 nm) within 30 minutes.**



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