THERATEST LABS
Innovations in Immunoassays

EL-Glia™ IgA/IgG

An enzyme immunoassay
for the detection and measurement
of anti-gliadin antibodies of IgA and IgG isotype

For professional use only

Instruction Manual

Catalog No.:

104-118

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INTRODUCTION

Name: EL-Glia™ IgA/IgG

IVD

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 familiar 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 Sjogren'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 (tTG) 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 first-line diagnostic test and as a tool for population screening (20).

The TheraTest EL-Glia™ IgA/IgG test system detects both IgA and IgG class anti-gliadin antibodies. Generally, the specificity of IgA anti-gliadin antibodies exceeds that of IgG

antibodies, while the latter tends to be more sensitive. Patients with IgA deficiency produce IgG antibodies only, usually in high amount (17). The antibody titer 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-Glia™ 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 gliadin. Wells are coated with purified gliadin, and incubated with Specimens, Calibrators, Positive and Negative Controls. During the incubation, anti-gliadin 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 gliadin 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

IVD

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://bmbl.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) (≤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 reagents.
6. Avoid exposing reagents to excessive heat or light during storage.
7. Chromogen contains an organic solvent that irritates eyes and mucus membranes.
8. Do not allow the Chromogen to come in contact with metal or oxidizing agents.
9. Use disposable glassware and plasticware or wash all material thoroughly according to standard laboratory practice.
10. tTG/Gliadin 10X Wash Buffer, Chromogen and Stop reagent are interchangeable among 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 $2^{o} – 8^{o}$C when received. Avoid freezing reagents.
2. All reagents must be brought to room temperature ($18^{o} – 25^{o}$C) for 30 minutes prior to use.
3. Avoid direct sunlight.

IMPORTANT: When stored at 2º - 8ºC, 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 2º - 8ºC, 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 2º - 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 gliadin. 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 2º - 8°C.
2. **tTG/Gliadin 10X Wash Buffer, 100 mL:** 10X concentrated buffer with Tween 20 & preservative.
3. **IgA Calibrator, 1.5 mL*:** Contains human serum with IgA antibodies to gliadin, and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
4. **IgG Calibrator, 1.5 mL*:** Contains human serum with IgG antibodies to gliadin and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
5. **IgA Positive Control, 0.35 mL*:** Human serum containing IgA antibodies to gliadin and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
6. **IgG Positive Control, 0.35 mL*:** Human serum containing IgG antibodies to gliadin, and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
7. **Negative Control, 0.35 mL*:** Human serum without IgA and IgG antibodies to gliadin and preservative in stabilizing buffer. See attached 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 (Fcγ specific) conjugated with horseradish peroxidase, with preservative and green dye.
10. **Chromogen, 27 mL:** 3,3'5,5' teramethybenzidine (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 µL, 100 µL 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).

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 attached Data Sheet. The entire strip (or strips) may be employed, or individual wells may be used as desired.

2. Wash Solution

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 2º - 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 µL 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 as prediluted. They are ready to use.

Assay Procedure

1. Allow all reagents and patient sera to equilibrate to room temperature prior to use (18º-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 attached 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 (18º - 25 °C).
8. Aspirate or decant the content 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 (18º - 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 (18º - 25 °C).
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 plate 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: A 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 should be run in each assay. The Positive and Negative Control values should fall within the ranges provided on the enclosed Data Sheet. If the values are not in agreement with those on 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 – 0.50 = 1.100

Note: If the absorbance in the Diluent 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:

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

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

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

Test	Normal	Equivocal	Abnormal
Anti-gliadin IgA	≤ 20 U/mL	21-25 U/mL	> 25 U/mL
Anti-gliadin 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-Glia™ IgA/IgG assays should not be performed on grossly hemolyzed, lipemic, icteric or microbially contaminated samples. This method has been tested using serum samples only. The performance using other types of specimens has not been determined.
3. 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).
4. This assay has not been evaluated on a pediatric population.

EXPECTED VALUES

The prevalence of anti-gliadin 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-gliadin antibodies in celiac disease (23-26).*

	Sensitivity *	Specificity *
Anti-gliadin IgA	52-91%	67-95%
Anti-gliadin IgG	62-83%	82-92%

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

IgA anti-gliadin antibodies are more specific diagnostic markers for CD than IgG antibodies. However, IgA deficient subjects produce only IgG antibodies. As gliadin is a food component, a

few percentages of healthy people can develop antibodies against it, without having gluten sensitive enteropathy.
The level of anti-gliadin antibodies 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-tTG antibodies 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-Glia™ IgA/IgG* assay. The results are presented in **Tables 2A and 2B**.

Table 2. *Frequency of (A) anti-gliadin IgA and (B) anti-gliadin IgG antibodies measured by the TheraTest EL-Glia™ 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-Glia™ IgA	**Positive**	24	4	28
	Equivocal	3	4	7
	Negative	17**	148	165
	Total	44	156	200

* The group includes patients on gluten-containing and gluten-restricted diet, as well.
**Two patients in this group are IgA deficient

Sensitivity: 55% (24/44)
Specificity: 95% (148/156)

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-Glia™ IgG	**Positive**	24	6	30
	Equivocal	5	4	9
	Negative	15	146	161
	Total	44	156	200

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

Sensitivity: 55% (24/44)
Specificity: 94% (146/156)

PERFORMANCE CHARACTERISTICS

Comparative studies

a) 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-Glia™ IgA/IgG* assay and another commercially available anti-gliadin IgA and IgG ELISA. The results are presented in **Tables 3**. and **4.**

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

		Other ELISA		
		Positive	**Negative**	**Total**
EL-Glia™ IgA	**Positive**	21	3	24
	Equivocal*	(2)	(1)	(3)
	Negative	1	78	79
	Total	22	81	103

*Equivocals were excluded from the calculation.

Positive agreement: 21/22; 95% (95% CI: 77%-100%)
Negative agreement: 78/81; 96% (95% CI: 90%-99%)
Total agreement: 99/103; 96% (95% CI: 90%-99%)

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

		Other ELISA		
		Positive	**Negative**	**Total**
EL-Glia™ IgG	**Positive**	29	5	34
	Equivocal*	(6)	(1)	(7)
	Negative	7	58	65
	Total	36	63	99

*Equivocals were excluded from the calculation.

Positive agreement: 29/36; 81% (95% CI: 64%-92%)
Negative agreement: 58/63; 92% (95% CI: 82%-97%)
Total agreement: 87/99; 88% (95% CI: 80%-94%)

Cross-reactivity, i.e. specificity based on disease controls
Testing was performed with 56 sera consisting of specimens from 40 inflammatory bowel disease (IBD) patients 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-Glia™ IgA assay*

A)

Anti-gliadin IgA level (U/mL)	**Intra-assay CV%**
154	7.6
65	5.8
29	4.0

B)

Anti-gliadin IgA level (U/mL)	**Inter-assay CV%**
134	8.4
60	8.6
34	10.1

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

A)

Anti-gliadin IgG level (U/mL)	**Intra-assay CV%**
88	2.4
58	6.0
23	10.8

B)

Anti-gliadin IgG level (U/mL)	**Inter-assay CV%**
65	9.0
44	9.3
29	10.2

TROUBLESHOOTING

Problem	Possible Causes	Solution
Control values out of range.	1. Incorrect temperature, timing or pipetting; reagents not mixed.	1. Check that temperature was correct. Check that time was correct. See "Poor Precision" (below) No. 2-4. Repeat test.
	2. Cross-contamination of controls.	2. Pipette carefully.
	3. Improper dilution.	3. Repeat test.
	4. Optical pathway not clean.	4. Check for moisture or dirt. Wipe bottom and reread.
	5. Wavelength of filter incorrect.	5. Change filter to 450 ± 5 nm.
	6. Specimen diluent blank >0.150	6. Check for contamination of Chromogen or conjugate solution.
All test results negative.	1. One or more reagents not added, or added in wrong sequence.	1. Recheck procedure. Check for unused solutions. Repeat test.
	2. Improper dilution of wash buffer.	2. Repeat test.
	3. Antigen coated plate inactive.	3. Check for obvious moisture in unused wells. Rerun test with controls only for activity.
All test results yellow	1. Contaminated chromogen.	1. Check absorbance of unused chromogen.
Scattered false positives	2. Contaminated buffers/reagents	2. Check all solutions for turbidity.
	3. tTG/Gliadin 1X Wash Buffer contaminated.	3. Use clean container for 1X Buffer. Check quality of water used to prepare buffer.
	4. Improper dilution of serum.	4. Repeat test.
	5. Contaminated pipette	5. Use felt-plugged tips for chromogen
Poor precision.	1. Pipettor delivery CV greater than 5%.	1. Check calibration of pipettor. Use reproducible technique.
	2. Serum or reagents not mixed sufficiently; reagents not at room temperature prior to addition.	2. Mix all reagents gently but thoroughly and equilibrate to room temperature.
	3. Reagent addition taking too long; inconsistency in timing intervals, air bubbles.	3. Develop consistent uniform technique and avoid splashing or use multi-channel device or autodispenser to decrease time.
	4. Air currents blowing over plate during incubations.	4. Cover plate or place in chamber.
	5. Optical pathway not clean.	5. Wipe bottom of plate with soft tissue. Check instrument light source and detector for dirt.
	6. Instrument not equilibrated before readings were taken.	6. Check instrument manual for warm up procedure.
	7. Washing not consistent; trapped bubbles; liquid left in wells at end of wash cycle.	7. Use only acceptable washing devices. Lengthen timing delay on washing devices. Check that all wells are filled.
	8. Improper pipetting.	8. Avoid air bubbles in pipette tips.

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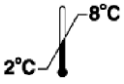

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EL-Glia™ IgA/IgG Abbreviated Test Procedure

1. **Dilute Controls and Specimens 1:101 with tTG/Gliadin 1X Wash Buffer.**
2. **Pipette 100 µL 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 (18º - 25ºC).**
4. **Wash the wells three times with tTG/Gliadin 1X Wash Buffer.**
5. **Add 100 µL of the Enzyme Conjugates (IgA or IgG) into appropriate wells.**
6. **Incubate for 30-35 minutes at room temperature (18º - 25ºC).**
7. **Wash the wells three times with tTG/Gliadin 1X Wash Buffer.**
8. **Add 100 µL of Chromogen into each well.**
9. **Incubate for 15±1 minutes at room temperature (18º- 25ºC).**
10. **Add 100 µL 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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