tTG/DGP ELISA KIT

Cat. No.:DEIA05755
Pkg.Size:96T

Intended use

Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of anti-human Tissue Transglutaminase (tTG) and deamidated gliadin peptide (DGP) IgA and IgG antibodies in human serum to aid in the diagnosis of gluten sensitive enteropathy / celiac disease in conjunction with other laboratory and clinical findings.

General Description

Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Studies have found the prevalence of CD to be highly variable. The current estimated prevalence of CD is 1%, with a statistical range of probability between 0.5–1.26% in the general population in Europe and the USA. Using serological methods, the incidence of CD in the general population is approximately one in 100.

Failure to diagnose CD early on may predispose an individual to long-term complications such as splenic atrophy and intestinal lymphoma. A gluten-free diet normalizes the mucosa and helps reduce the malignant potential. Histological examination of the small intestinal biopsy remains the gold standard for diagnosing CD, but it has its own limitations. These include some patients with latent or even active CD that may have normal histopathology.

The various serological tests employed in the work-up of patients suspected to have CD include gliadin (AGA), DGP, tTG and endomysial (EMA), antibody tests. Antibodies to gliadin, DGP, and tTG are detected by ELISA, whereas EMA are detected by indirect immunofluorescence. EMA are very specific indicators of CD. However, the EMA test is an immunohistochemical method that requires experience in reading immunofluorescence reactions.

Principle Of The Test

The test is performed as a solid phase immunoassay. Microwells are coated with a recombinant antigen containing tTG and DGP epitopes followed by a blocking step to reduce non-specific binding during the assay run. Controls, calibrators and patient sera are incubated in the antigen coated wells to allow specific antibodies present in the serum to bind to the antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgA/IgG conjugate to the microwells. Unbound conjugate is removed by washing. Specific enzyme substrate (TMB) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of TMB substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/ml) and reported as positive or negative.

Reagents And Materials Provided

1 x 1.75 ml [CONTROL|+] Ready to use Positive Control (red cap). Contains human serum positive for tTG and DGP antibodies. The expected concentration range in EU/ml is printed on the label.
1 x 1.75 ml [CONTROL|-] Ready to use Negative Control (white cap). Contains human serum.
5 x 1.75 ml [CALIBRATOR|A|FUSION] [CALIBRATOR|B|FUSION] [CALIBRATOR|C|FUSION] [CALIBRATOR|D|FUSION] [CALIBRATOR|E|FUSION]

Ready to use set of 5 Calibrators. Calibrator A (green cap) 160 EU/ml, Calibrator B (violet cap) 80 EU/ml, Calibrator C (blue cap) 40 EU/ml, Calibrator D (yellow cap) 20 EU/ml, and Calibrator E (orange cap) 1 EU/ml. Derived from human serum containing tTG and DGP antibodies. Concentrations in EU/ml are printed on the labels.

1 x 15 ml [SUBSTRATE|TMB] TMB enzyme substrate. Ready for use. Protect from light.
2 x vials [BUF|WASH] Powder Wash Buffer. Reconstitute to one liter each.
1 x Protocol Sheets

Optional Components
1 x 60ml [BUF|WASH] Liquid concentrated Wash Buffer. Reconstitute to one liter.

Materials Required But Not Supplied

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettes capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper towels
- Microplate reader capable of reading absorbance values at 450 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm
- Automatic microplate washer capable of dispensing 200 µl

Storage

Store all reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.

Specimen Collection And Handling

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2°- 8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples. It is recommended that frozen specimens be tested within one year.

Plate Preparation

Coated microwell strips are for one time use only. Unused microwell strips should be carefully resealed in the pouch containing desiccants to prevent condensation and stored at 2-8°C.

Reagent Preparation

Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use.
Reconstitute the wash buffer to 1 liter with distilled or deionized water. When stored at 2-8°C, the reconstituted wash buffer is
stable until the kit expiration date.

Assay Steps

Procedural Notes
• Carefully read the product insert before starting the assay.
• All dilutions of the patient samples should be prepared prior to starting with the assay.
• Let patient specimens and test reagents equilibrate to room temperature before starting with the test procedure. It is suggested that reagents be left on the bench out of the box for 30 minutes prior to use. Return all unused specimens and reagents to refrigerator immediately after use.
• Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.
• Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. An automated microplate washer is recommended.
• Use a multichannel pipette capable of delivering 8 or 12 wells simultaneously. This speeds the process and provides more uniform incubation times.
• For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
• Addition of all samples and reagents should be performed at the same rate and in the same sequence.

Test Method
Step 1 Let all reagents and specimens equilibrate to room temperature.
Step 2 Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.
Step 3 For a qualitative determination use Calibrator D (vial with yellow cap) only.

or
For a semi-quantitative determination use Calibrators A through E as depicted in the sample layout below.

<table>
<thead>
<tr>
<th>Qualitative</th>
<th>Semi-Quantitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Blank S5</td>
<td>A Blank S1 Etc.</td>
</tr>
<tr>
<td>B - S6</td>
<td>B - S2</td>
</tr>
<tr>
<td>C + S7</td>
<td>C + S3</td>
</tr>
<tr>
<td>D Cal D S8</td>
<td>D Cal A S4</td>
</tr>
<tr>
<td>E S1 S9</td>
<td>E Cal B S5</td>
</tr>
<tr>
<td>F S2 S10</td>
<td>F Cal C S6</td>
</tr>
<tr>
<td>G S3 S11</td>
<td>G Cal D S7</td>
</tr>
<tr>
<td>H S4 S12</td>
<td>H Cal E S8</td>
</tr>
</tbody>
</table>

Step 4 Prepare a 1:101 dilution of the patient samples by mixing 5 µl of the patient sera with 500ul of Serum Diluent.
Step 5 Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder.
Step 6 Pipette 100 µl of Ready to use Calibrators, Positive and Negative controls and diluted patient samples (1:101) to the
appropriate microwells as per protocol sheet.

Note: Include one well which contains 100 µl of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank.

Step 7 Incubate 30 minutes (± 5 min) at room temperature.

Step 8 Wash 4x with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer’s instructions.

Step 9 Pipette 100 µl of Conjugate into microwells.

Step 10 Incubate 30 minutes (± 5 min) at room temperature.

Step 11 Wash all microwells as in Step 8.

Step 12 Pipette 100 µl of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 13 Incubate 30 minutes (± 5 min) at room temperature.

Step 14 Pipette 100 µl of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance values within 30 minutes of adding Stop Solution.

Step 15 Read absorbance of each microwell at 450 nm using a single or at 450/630nm using a dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. The Calibrator A should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The negative control must be <10 EU/ml. If the test is run in duplicate, the mean of the two readings should be taken for determining EU/ml. While performing Qualitative determinations, the optical density of Calibrator D must be greater than that of the negative control and less than the absorbance of the positive control.

For semi-quantitative determinations the positive control must give values in the range stated on the vial.

Calculation

The concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

\[(\text{Abs. of Test Sample}/\text{Abs. of Calibrator D}) \times \text{EU/ml of Calibrator D} = \text{EU/ml Test Sample}\]

It is recommended that qualitative results be reported as “positive” or “negative.” Sample results greater than or equal to Calibrator D are considered positive.

2. SEMI-QUANTITATIVE DETERMINATION

Plot the absorbance of Calibrator A through E against their respective concentrations on linear-log graph paper. Plot the concentrations in EU/ml on the X-axis against the absorbance on the Y-axis and draw a point-to-point curve fit. Determine the concentrations of the patient samples from the curve according to corresponding absorbance values. Alternately, a four parameter curve may be used to plot the standard curve.

It is recommended that semi-quantitative results be reported as “positive,” “negative,” or “indeterminate” with EU/ml unit values. Indeterminate/borderline results should be retested and evaluated along with other laboratory methods, such as assays for detection of EMA and/or Gliadin antibodies.

Evaluation

Test results in a normal population are usually expected to be negative. However, as the incidence of CD in the normal population is about 1%, some apparently healthy, asymptomatic individuals may test positive for tTG antibodies.
The incidence and levels of autoantibodies associated with gluten sensitive enteropathy / CD are dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients who are on a gluten-free diet. Similarly, the levels of these antibodies will increase and may become positive when patients with CD who were on a gluten-free diet ingest a gluten-containing diet. Patients who have CD but are IgA deficient will generally be positive for IgG antibodies to tTG and gliadin. In such cases studies can be performed to confirm that the patient is IgA deficient.

Sets of clinical samples were tested on the Celiac Fusion ELISA. Results demonstrating incidence in the populations for this study are provided below.

Note: Population tested included a large number of challenging low EMA titer samples and does not reflect a typical diagnosed CD population nor a typical screening population.

### Interpretation of Results

The following serves only as a guide in the interpretation of the laboratory results. These values were determined by testing 123 normal blood donors and non-celiac disease control specimens. The mean of the normal subjects plus 2.5 SD was established as the assay cutoff and assigned an arbitrary value of 20 EU/ml. Each laboratory must determine its own normal values.

<table>
<thead>
<tr>
<th>Celiac Fusion value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20 EU/ml</td>
<td>Negative</td>
</tr>
<tr>
<td>20-25 EU/ml</td>
<td>Indeterminate (Borderline)</td>
</tr>
<tr>
<td>&gt;25 EU/ml</td>
<td>Positive</td>
</tr>
</tbody>
</table>

#### Sensitivity

The limit of detection (LoD) was determined based on 60 replicates of the blank and 10 replicates each of 6 low-level (NHS) samples. LoD for was 3.0 EU/ml.

#### Specificity

The utility of the Celiac Fusion™ ELISA was evaluated by testing well-characterized EMA positive serum specimens from CD
subjects alongside disease controls and “normal” human sera. These specimens were also tested on a commercially available tTG/DGP Antibody Screen ELISA. These results are summarized below.

A. Method Comparison: Celiac Fusion ELISA vs. other tTG/DGP screen ELISA.

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMMCO Positive</td>
<td>52</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>Celiac Fusion™</td>
<td>2</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>Screen ELISA Total</td>
<td>54</td>
<td>55</td>
<td>109</td>
</tr>
</tbody>
</table>

Positive Percent Agreement: 96.3% (95% CI 86.2% to 99.4%)
Negative Percent Agreement: 94.5% (95% CI 83.9% to 98.6%)
Overall Percent Agreement: 95.4% (95% CI 89.1% to 98.3%)

EMA Positive Celiac Subjects: 57
Disease Controls: 20
Healthy Normal Subjects: 32

B. Cross Reactivity: A total of 64 potentially cross-reactive specimens from individuals with other autoimmune disorders or positive for other autoantibodies were tested for tTG/DGP antibodies using the Celiac Fusion ELISA.

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>n Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hashimoto’s Thyroiditis</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Mixed Connective Tissue Disease</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>1 (1.6%)</td>
</tr>
</tbody>
</table>

**Linearity**

**Linearity and Recovery**

Linearity and recovery were tested by diluting positive specimens through the assay range in equidistant dilutions and comparing actual vs. expected results. The linear range of the assay was determined be 3.0 (LoD) – 160 EU/ml. Results are summarized below:
Accuracy

Precision was tested with multiple specimens selected throughout the range of the assay. Multiple assay runs were performed to determine results between days. An additional run of twelve replicates was performed to determine repeatability. Results are summarized below.

Reproducibility

Eighty replicates of samples in the negative range, ~20% below cutoff, ~20% above cutoff and in the moderate positive range of the assay were performed to determine qualitative reproducibility. These samples were tested in multiple runs. Assay results for each specimen produced 100% qualitative agreement.

Interferences

Interference was studied by mixing sera with known iTG/DGP antibody levels with potentially interfering serum samples and studying deviation from expected results. No significant interference was demonstrated for the following substances at the levels indicated: Hemoglobin (2 g/L), Bilirubin (342 μmol/L), Rheumatoid Factor (100 EU/ml), Triglycerides (37 mmol/L) and Cholesterol (130 mmol/L).

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious.

Follow good laboratory practices in storing, dispensing and disposing of these materials. Stop Solution is a dilute sulfuric acid solution. Sulfuric acid (H2SO4) is poisonous and corrosive. Do not ingest and avoid contact with skin and eyes. Avoid exposure to bases, metals or other compounds that may react with acids.

TMB Enzyme Substrate contains an irritant that may be harmful if inhaled, ingested or absorbed through the skin. Do not ingest and avoid contact with skin and eyes.
Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use kit components beyond expiration date on the labels.

Limitations

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples. Test results serve as an aid in the diagnosis and should be considered in conjunction with other laboratory and clinical findings.