EliA uses a modular reagent system. All information needed to understand the use of the EliA tests can be found in the analyte specific DfU and the corresponding EliA Control DfU on this CD.

CONTENTS

Summary and Explanation of the Test

Celiac disease is a life-long condition in which ingestion of gluten, the water insoluble wheat-gliadin and the prolamins in rye and barley, leads to chronic inflammation and damage of the small intestinal mucosa. The disease is multifaced in nature with clinical presentation ranging from gastrointestinal manifestations to asymptomatic, silent and extraintestinal forms.\(^1\) It is widely accepted that dermatitis herpetiformis, a bullous skin disease, is induced by gluten.\(^2\) The term gluten refers to a whole set of proteins in the so-called endosperm, the nutritive tissue of the grain seed of wheat, rye, oats and barley. The alcohol-soluble polypeptides of gluten, the gliadins, are solely responsible for the toxic effects to the intestinal mucosa.\(^3\) Since the levels of gliadin antibodies correlate well with the morphological appearance of the small-intestinal mucosa, the non-invasive serological diagnosis of celiac disease is increasingly replacing the biopsy procedure, especially in children.\(^4,5,6,7,8\)

Principles of the Procedure

The EliA Gliadin IgG Wells are coated with gliadin antigen. If present in the patient’s specimen, antibodies to gliadin bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

Reagents / Material

The EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA Celiac Positive Control 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA Gliadin IgG Test. The EliA Gliadin IgG Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.
**EliA Gliadin IgG Curve Control Strips (Art. No 83-1016-01)**

- **human IgG (20 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CC-1**
- **5 strips**
- **Each strip contains 6 x 0.3 ml**
- **CC-1 (double determination)**
- **ready for use; store at 2-8 °C until expiration date**

Manufactured from human sera.

**EliA Gliadin IgG Calibrator Well (Art. No 14-5509-01)**

- **IgG Calibrator Well coated with mouse monoclonal antibodies; short name: Gc**
- **4 carriers (12 wells each); sufficient for 48 determinations**
- **ready for use; store dry at 2-8 °C until expiration date**

**Phadia 250 General Reagents**

**Development Solution (Art. No. 10-9440-01)**

- **Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative**
- **6 bottles (17 ml each); sufficient for 6 x >170 determinations**
- **ready for use; store at 2-8 °C until expiration date**

**Stop Solution (Art. No. 10-9442-01)**

- **Stop Solution 4 % Sodium Carbonate**
- **6 bottles (119 ml each); sufficient for 6 x >560 determinations**
- **ready for use; store at 2-8 °C until expiration date**

**Dilution Plates (Art. No. 12-3907-08)**

- **MicroWell™ plates with 96 wells, 0.5 ml each; Polypropylene**
- **60 plates per package; sufficient for 60 x 96 samples**
- **ready for use**

*Preservative: mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-3] (3:1).*

**Washing Solution (Art. No. 10-9422-01/10-9202-01)**

For information see separate Washing Solution package insert.

**WARNINGS AND PRECAUTIONS**

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Wear gloves while handling samples and reagents provided.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

**WARNING!** Reagents contain sodium azide (NaNO₂) as a preservative. NaNO₂ may be toxic if ingested or absorbed by skin or eyes. NaNO₂ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local and national guidelines. Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

**Indication of Instability**

Phadia 250 Instrument Software has built-in acceptance limits for the calibration curve and the curve control. EliA Wells are moisture sensitive. An activity loss that might occur due to inappropriate handling can be detected using the appropriate EliA Control. For more information see Phadia 250 User's Guide/Reference Manual.

**INSTRUMENT**

EliA reagents are to be used with the Phadia 250 Instrument Software version 1.06 or higher. The Phadia 250 Instrument processes all steps of the test. For further information regarding test set-up, instrumentation and software etc. see Phadia 250 User's Guide/Reference Manual.

**SPECIMEN COLLECTION, HANDLING AND PREPARATION**

The procedure can be performed with serum or plasma specimens. Lipemic, hemolyzed or microbially contaminated samples may give poor results and should not be used. Avoid repeated freezing and thawing. Samples should be stored in aliquots at -20 °C (-4 °F) or below for repeated measurements.

**Sample Dilution**

Samples must be diluted with EliA Sample Diluent. A 1:100 dilution of the samples is required for the EliA Gliadin IgG Test. Samples can be diluted manually, but instrument dilution is recommended and is a default setting in Phadia 250 Instrument Software.

**PROCEDURE**

**Handling of EliA Gliadin IgG Well**

In the Phadia 250 storage chamber, carriers are stable for up to 28 days. If you are not expecting to use them up within this time, the carriers should be loaded via the Phadia 250 Loading Tray and, for stability reasons, must be put back into the desiccant-containing foil bag directly after the run. Because it is important to store the wells in dry conditions at 2-8°C, the bag must be properly resealed. If stored under these conditions, the shelf-life from the date of first opening is 9 months, if not limited by the expiry date stated on the carrier and foil bag.

**Lot specific barcode**

Use the built-in barcode reader to enter the lot specific information of EliA Gliadin IgG Well, EliA IgG Calibrator Well and EliA IgG Conjugate. In case of manual handling make sure to enter the characters below the barcode.

**On-board stability of reagents**

- **EliA Wells**
  - EliA Well carriers can be stored on-board for 28 days at 2-8°C or 24 hours at room temperature.
- **EliA Calibrator Strips, EliA Curve Control Strips**
  - Can be stored on-board for 28 days. During longer interval between usage (>3 days) it is recommended to unload the strips from the instrument and store them at 2-8°C.
- **EliA Sample Diluent**
  - Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.
**Gliadin IgG**

**Panel Description**
- EliA tests included
- pceli Celiac disease
- pcdg Celiac disease IgG
- pgli Gliadin

**CALIBRATION AND REFERENCE MATERIAL**

The calibration curve is obtained with EliA IgG Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA IgG Curve Control (run in duplicate). The IgG Calibrators are traceable via an unbroken chain of calibrations to the International Reference Preparation (IRP) 67/86 of Human Serum Immunoglobulins A, G and M from World Health Organization (WHO).

A new calibration curve must be run when:
- the last calibration was made more than one month ago or
- a new lot of EliA IgG Conjugate is introduced or
- when the EliA IgG Curve Control is outside the specified limits (defined in Phadia 250 Instrument Software).

There are no international standards for Gliadin antibodies. Results are given in arbitrary EliA Units/ml.

**QUALITY CONTROL**

**Control Specimens**

Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptance ranges.

EliA Controls are available for the quality control of the measurements.

**CALCULATION AND INTERPRETATION OF RESULTS**

**Presentation of Results**

Phadia 250 measures specific IgG concentrations in μg/l. By using a conversion factor given by the lot-specific code of the EliA Gliadin IgG Well, the results are automatically converted to EliA U/ml.

**Interpretation of Test Results**

The ranges (negative, equivocal, positive) recommended for the evaluation of the results are given in the table below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>negative</th>
<th>equivocal</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Gliadin IgG</td>
<td>EliA U/ml</td>
<td>&lt; 7</td>
<td>7 - 10</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>

Good laboratory practice requires that each laboratory establishes its own range of expected values.

**LIMITATIONS**

A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

**EXPECTED VALUES**

Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for gliadin antibodies...
Results Obtained for Healthy Subjects
The frequency distribution for Gliadin antibodies was investigated in a group of apparently healthy subjects equally distributed by age and gender, using sera from a Caucasian population obtained from a blood bank. The results are given in the table below.(1)

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>No. of Samples</th>
<th>Mean Value</th>
<th>95%- percentile</th>
<th>99%- percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Gliadin IgG</td>
<td>EliA U/ml</td>
<td>400</td>
<td>1.7</td>
<td>5.0</td>
<td>12.6</td>
</tr>
</tbody>
</table>

PERFORMANCE CHARACTERISTICS

Measuring Range
The measuring range (detection limit, upper limit) for EliA Gliadin IgG is from 0.2 to \( \geq 192 \) EliA U/ml. No hook effects could be observed for concentrations up to 13 fold above the measuring range.(1)

Only values above the Detection Limit can be regarded as valid results. The upper limit of the reported results can vary due to a lot-specific conversion from \( \mu g/l \) to EliA U/ml. Results above the upper limit are reported as “above”.

Please note that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range.

Specificity
The EliA Gliadin IgG Test permits the determination of IgG antibodies directed against the gliadin antigen as described in section “Reagents”.

Precision
To determine the precision of the assay, the variability was assessed in studies with 13 runs by examining 3 samples on 3 instruments. The statistical evaluation was performed by Analysis of Variance. The results are given in the table below.(2)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>Unit</th>
<th>Mean Value</th>
<th>Coefficients of variation (%)</th>
<th>Intra-Run</th>
<th>Inter-Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Gliadin IgG</td>
<td>1</td>
<td>EliA U/ml</td>
<td>10.1</td>
<td>4.6</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>EliA U/ml</td>
<td>31.9</td>
<td>3.7</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>EliA U/ml</td>
<td>93.7</td>
<td>3.8</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES

WARRANTY
The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Phadia AB and its authorized distributors, in such event, shall not be liable for damages, indirect or consequential.

LOT

Batch code

Contains sodium azide

Biological Risk

Contains x determinations

Store at 2-8°C/35-46°F

Read Directions for Use

Expiration date

Manufactured by

For in vitro diagnostic use

Do not reuse in a second run

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