Intrinsic Factor

**CONTENTS**

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.

**INTENDED USE**

EliA Intrinsic Factor is intended for the in vitro quantitative measurement of IgG antibodies directed to intrinsic factor in human serum and plasma to aid in the clinical diagnosis of pernicious anemia.

EliA Intrinsic Factor uses the EliA IgG method on the instrument Phadia 250.

**SUMMARY AND EXPLANATION OF THE TEST**

Pernicious anemia (also known as Biermer’s disease) is an autoimmune atrophic gastritis, predominantly of the fundus, and is responsible for a deficiency in vitamin B12 (cobalamin) due to its malabsorption. Its prevalence is 0.1% in the general population and 1.9% in subjects over the age of 60 years. Pernicious anemia represents 20%–50% of the causes of vitamin B12 deficiency in adults.

Anti intrinsic factor antibodies do not appear to have a clearly defined pathogenic role in the development of gastritis. By contrast, they have a well-documented role in the onset of pernicious anemia, via the vitamin B12 deficiency they induce. ¹

The finding of a low total serum cobalamin level may be further evaluated by testing for anti intrinsic factor antibodies. If positive, the antibodies have a high predictive value (95%) for the presence of pernicious anemia with a concurrent low false positive rate (1–2%) i.e. a high specificity. It identifies those patients with a need for lifelong cobalamin replacement therapy.²

With regard to diagnostic performance sensitivity is low for anti intrinsic factor antibodies, in the most recent studies while specificity is 100%. In combination with anti parietal cell antibodies they yield 73% sensitivity and 100% specificity.¹

**PRINCIPLES OF THE PROCEDURE**

The EliA Intrinsic Factor Wells are coated with human gastric intrinsic factor. If present in the patient’s specimen, anti intrinsic factor antibodies bind to the 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 Gastric Positive Control 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA Intrinsic Factor Test.

The EliA Intrinsic Factor Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

---

**EliA Intrinsic Factor Test-Specific Reagents**

**EliA Intrinsic Factor Well (Art. No. 14-5668-01)**

| Intrinsic Factor Well; short name:inf | coated with human gastric intrinsic factor | 2 carriers (12 wells each); sufficient for 24 determinations | ready for use; store dry; until expiration date |

**EliA Gastric Positive Control 250 (Art. No 83-1145-01)**

| Human serum and human recombinant antibody in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos | Multiparameter control containing IgG antibodies to intrinsic factor and parietal cells | 6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial | Ready for use; store at 2-8 °C until expiration date |

**EliA IgG/IgM/IgA Negative Control 250 (Art. No 83-1037-01)**

| Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: neg | Multiparameter control containing normal sera from healthy donors | 6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial | ready for use; store at 2-8 °C until expiration date |

**EliA Sample Diluent (Art. No 83-1023-01)**

| Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095 %) | 6 bottles (48 ml each); sufficient for ≥6 x 180 dilutions | ready for use; store at 2-8 °C until expiration date |

**EliA IgG Conjugate 50 (Art. No 83-1017-01)**

| IgG Conjugate (blue colored); β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-G | 6 wedge shaped bottles (5 ml each); sufficient for 6 x 50 determinations | ready for use; store at 2-8 °C until expiration date; DO NOT FREEZE; DO NOT REUSE |

**EliA IgG Conjugate 200 (Art. No 83-1018-01)**

| IgG Conjugate (blue colored); β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-G | 6 wedge shaped bottles (19 ml each); sufficient for 6 x 200 determinations | ready for use; store at 2-8 °C until expiration date; DO NOT FREEZE; DO NOT REUSE |

**EliA IgG Calibrator Strips (Art. No 83-1015-01)**

| Human IgG (0, 4, 10, 20, 100, 600 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) | 5 strips | 6 single-use vials per strip (0.3 ml each); sufficient for one calibration curve (double determination) | ready for use; store at 2-8 °C until expiration date |

Manufactured from human sera.
Phadia 250 General Reagents

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

- Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative*
- Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative*

**Stop Solution (Art. No. 10-9422-01)**

- Stop Solution 4 % Sodium Carbonate

**Stop Solution (Art. No. 10-9479-01)**

- Stop Solution 4 % Sodium Carbonate

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

- MicroWell™ plates with 96 wells, 0.5 ml each; Polypropylene

**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.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunosassay 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 (NaNO₃) as a preservative. NaN₃ may be toxic if ingested or absorbed by skin or eyes. NaN₃ 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**

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.

- Undiluted samples should remain at room temperature for no longer than eight hours.*
- Undiluted samples can be stored at 2-8°C for two weeks without degradation provided they do not become contaminated by bacteria or fungi and they should be frozen at below -20°C for any long-term storage.**

Note: It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory. In general, laboratories should perform validation studies before implementing a change in specimen acceptance criteria.*


**Sample Dilution**

Samples must be diluted with EliA Sample Diluent. A 1:100 dilution of the samples is required for the EliA Intrinsic Factor Test. Samples can be diluted manually, but instrument dilution is recommended.

**PROCEDURE**

**Handling of EliA Intrinsic Factor 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 until expiration date DO NOT FREEZE.

**Procedure**

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.

1. **Undiluted samples should remain at room temperature for no longer than eight hours.***
2. **Undiluted samples can be stored at 2-8°C for two weeks without degradation provided they do not become contaminated by bacteria or fungi and they should be frozen at below -20°C for any long-term storage.**

**Note:** It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory. In general, laboratories should perform validation studies before implementing a change in specimen acceptance criteria.

3. **Sample Dilution**

   Samples must be diluted with EliA Sample Diluent. A 1:100 dilution of the samples is required for the EliA Intrinsic Factor Test. Samples can be diluted manually, but instrument dilution is recommended.

4. **PROCEDURE**

   **Handling of EliA Intrinsic Factor 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.

5. **Lot specific barcode**

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

6. **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.

   **EliA Sample Diluent**

   Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.
Intrinsic Factor

Sample volumes per determination

Manual dilution: 90 μl of diluted sample
Instrument dilution (1:100): 20 μl of non diluted sample


Reagent volumes per determination

Calibrator 90 μl
EliA IgG Conjugate 90 μl
Development Solution 90 μl
Stop Solution 200 μl

Procedural comments

• From one sample diluted by the instrument (1:100), up to 11 determinations can be made.
• When using software default, samples are run in single determination.
• Washing Solution must be at room temperature when used.
• The first result is available after approx. 2 hours and further results at one minute intervals afterwards. Up to 5 x 10 samples can be loaded continuously and are processed by random access.
• Incubations are automatically performed at 37 °C (98.6 °F).

There are no international standards for anti intrinsic factor 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 Intrinsic Factor 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 Intrinsic Factor</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. Early reports suggest that intrinsic factor antibodies are found in 40–60% of patients with pernicious anemia. The incidence of these antibodies have been reported to rise to about 60–80% with increasing duration of disease.\(^3\) Expected values may vary depending on the population tested.

Results Obtained for Healthy Subjects

The frequency distribution for anti intrinsic factor 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.

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>No. of samples</th>
<th>Median value</th>
<th>95-%tile</th>
<th>99-%tile</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Intrinsic Factor</td>
<td>EliA U/ml</td>
<td>400</td>
<td>1.1</td>
<td>1.9</td>
<td>2.9</td>
</tr>
</tbody>
</table>

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA Intrinsic Factor is from 0.5 to 480 EliA U/ml No hook effects could be observed for concentrations up to 34 fold above the measuring ranges. Only values above the Detection Limit can be regarded as valid results. Results above the upper limit are reported as >480. 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 Intrinsic Factor Test permits the determination of anti intrinsic factor antibodies directed against the antigen as described in section “Reagents”.

Precision

To determine the precision of the assay, the variability was assessed in a study with 21 runs by examining the samples in 252 replicates on 3 instruments over 7 days with a calibration curve in each run. The statistical evaluation was performed by Analysis of Variance. The results are given in the table below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>Unit</th>
<th>Mean value</th>
<th>Intra-Run</th>
<th>Inter-Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Intrinsic Factor</td>
<td>1</td>
<td>EliA U/ml</td>
<td>9.6</td>
<td>4.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>EliA U/ml</td>
<td>14.1</td>
<td>4.0</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>EliA U/ml</td>
<td>435.3</td>
<td>4.7</td>
<td>3.9</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.

REFERENCES