FLUORENZYME IMMUNOASSAY FOR ANTI CARDIOLIPIN ANTIBODIES

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 DU.

INTENDED USE

EliA Cardiolipin IgM is intended for the in vitro quantitative measurement of IgM antibodies directed to cardiolipin in serum and plasma to aid in the diagnosis of antiphospholipid syndrome (APS) and to evaluate the thrombotic risk in patients with systemic lupus erythematosus (SLE). EliA Cardiolipin IgM uses the EliA IgM method on the instrument Phadia 250.

SUMMARY AND EXPLANATION OF THE TEST

Anti-cardiolipin antibodies (ACA) belong to the group of anti-phospholipid antibodies (aPL). Their occurrence was first demonstrated in sera of syphilis patients, but later they have also been described frequently in SLE (systemic lupus erythematosus) patients (prevalence 30-40%) and in patients with other rheumatic diseases. The antiphospholipid syndrome (APS), also known as “Hughes syndrome”, is characterized by typical clinical features such as arterial/venous thromboses or recurrent miscarriages together with persistently positive tests for aPL. In contrast to “secondary APS” which occurs in association with SLE or other rheumatic disorders, there is no evidence for another relevant underlying disease in primary APS. New criteria for classification of the antiphospholipid syndrome have been defined recently.1,2,3,4,5,6,13 Anti-cardiolipin antibodies in infectious diseases and in APS can be distinguished with respect to their dependence on cofactors: whereas ACA from patients with infectious diseases recognize the pure phospholipid as antigen, binding of ACA from patients with APS requires β2-glycoprotein I as a cofactor. For this reason, ACA ELISAs need β2-glycoprotein I to be incorporated into the assay. The so-called ‘lupus anticoagulant’ (LA) describes a phenomenon that is related to the presence of antiphospholipid antibodies. It is defined by the measurement of antibody dependent coagulation inhibition in vitro.1,5,7,8,9 ACA/LA are considered to be of significant diagnostic relevance, as a correlation has been found between these antibodies and a tendency towards thromboses. This results in an increased incidence of venous/arterial thromboses (including apoplexy), thrombocytopenia, livedo reticularis, habitual abortion and neurological manifestations in ACA/LA-positive patients. Elevated levels of ACA/LA may also be found in patients with cerebrovascular insufficiency or myocardial infarction. aPL are discussed to play a direct role in the pathogenesis of APS.1,3,10,11,12,13

PRINCIPLES OF THE PROCEDURE

The EliA Cardiolipin IgM Wells are coated with bovine cardiolipin antigen. If present in the patient’s specimen, antibodies to cardiolipin bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgM antibodies (EliA IgM 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 IgM 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 APS Positive Control 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA Cardiolipin IgM Test. The EliA Cardiolipin IgM Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA Cardiolipin IgM Test-Specific Reagents

<table>
<thead>
<tr>
<th>EliA Cardiolipin IgM Well (Art. No. 14-5530-01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiolipin IgM Well; coated with bovine cardiolipin antigen and bovine β2-glycoprotein I as co-factor</td>
</tr>
<tr>
<td>4 carriers (12 wells each); sufficient for 48 determinations</td>
</tr>
<tr>
<td>ready for use; store dry at 2-8 °C until expiration date</td>
</tr>
</tbody>
</table>

EliA APS Positive Control 250 (Art. No 83-1055-01)

| Human monoclonal antibodies in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos |
| 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 |
| 6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial |
| EliA APS Positive Control 250 is prepared from human monoclonal antibodies. |

EliA Method-Specific Reagents (Phadia 250)

<table>
<thead>
<tr>
<th>EliA Sample Diluent (Art. No 83-1023-01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095 %)</td>
</tr>
<tr>
<td>6 bottles (48 ml each); sufficient for ≥6 x 180 dilutions</td>
</tr>
<tr>
<td>ready for use; store at 2-8 °C until expiration date</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EliA IgM Conjugate 50 (Art. No 83-1051-01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM Conjugate (blue colored); β2-Galactosidase anti-IgM (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-M</td>
</tr>
<tr>
<td>6 wedge shaped bottles (5 ml each); sufficient for 6 x 50 determinations</td>
</tr>
<tr>
<td>ready for use; store at 2-8 °C until expiration date</td>
</tr>
<tr>
<td>DO NOT FREEZE</td>
</tr>
<tr>
<td>DO NOT REUSE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EliA IgM Calibrator Strips (Art. No 83-1052-01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>human IgM (0.10, 35, 80, 500, 1000 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %)</td>
</tr>
<tr>
<td>6 single-use strips (0.3 ml each); sufficient for one calibrator curve</td>
</tr>
<tr>
<td>ready for use; store at 2-8 °C until expiration date</td>
</tr>
</tbody>
</table>

Manufactured from human sera.
**EliA Curve Control Strips (Art. No 83-1053-01)**

- Human IgM (80 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 Calibrator Well (Art. No 14-5527-01)**

- IgM Calibrator Well coated with mouse monoclonal antibodies; short name: Mcal
- 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


**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 (NaN₃) 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.

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**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 2.06 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. (Validation studies were performed using lithium heparin, citrate and EDTA plasma (1)). 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 1:10 dilution of the samples is required for the EliA Cardiolipin Test. Samples can be diluted manually, but instrument dilution is recommended.

**PROCEDURE**

**Handling of EliA Cardiolipin IgM 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. 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 Cardiolipin IgM Well, EliA IgM Calibrator Well and EliA IgM 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.
**Volumes per determination**

**Reagent volumes per determination**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>90</td>
</tr>
<tr>
<td>EliA IgM Conjugate</td>
<td>90</td>
</tr>
<tr>
<td>Development Solution</td>
<td>90</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>200</td>
</tr>
</tbody>
</table>

**Sample volumes per determination**

- Manual dilution: 90 μl of diluted sample
- Instrument dilution (1:10): 9 μl of non diluted sample


**Reagent volumes per 200 determinations**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing Solution</td>
<td>5 - 7*</td>
</tr>
<tr>
<td>Rinse Solution</td>
<td>5 - 6*</td>
</tr>
</tbody>
</table>

* The residual volume depends on the number of samples and dilution method used.

**Procedural comments**

- 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).

**CALIBRATION AND REFERENCE MATERIAL**

The calibration curve is obtained with EliA IgM Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA IgM Curve Control (run in duplicate).

The IgM 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 IgM Conjugate is introduced or
- when the EliA IgM Curve Control is outside the specified limits (defined in Phadia 250 Instrument Software).

The standardization of the assay is adjusted to a set of established standard sera (Harris et al., 1987). Results are expressed in MPL-U/ml (1 MPL-Unit corresponds to the binding activity of 1 μg/ml of cardiolipin IgM antibody that was purified from the standard serum by affinity chromatography).

**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 IgM concentrations in μg/l. By using a conversion factor given by the lot-specific code of the EliA Cardiolipin IgM Well, the results are automatically converted to MPL-U/ml.

**Interpretation of Test Results**

The ranges (negative, weak positive, 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>weak positive</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Cardiolipin IgM</td>
<td>MPL-U/ml</td>
<td>&lt; 10</td>
<td>10 - 40</td>
<td>&gt; 40</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. Rheumatoid factor (RF) can interfere with the determination of IgM anti-cardiolipin antibodies.

**EXPECTED VALUES**

Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for the Cardiolipin IgM antibodies covered by the EliA Cardiolipin IgM test is below 5 %4. Expected values may vary depending on the population tested.

**Results Obtained for Healthy Subjects**

The frequency distribution for Cardiolipin IgM antibodies was investigated on the instrument Phadia 100 in a group of apparently healthy subjects equally distributed by age and...
PERFORMANCE CHARACTERISTICS

Measuring Range
The measuring range (detection limit, upper limit) for EliA Cardiolipin IgM is from 0.8 to ≥ 472 MPL-U/ml. No hook effects could be observed for concentrations up to 10 fold above the measuring range. 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 μg/l to MPL-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 Cardiolipin IgM Test permits the determination of IgM antibodies directed against the Cardiolipin antigen as described in section “Reagents”.

Precision
To determine the precision of the assay, the variability was assessed in a study with 18 runs by examining the samples in 108 replicates on 3 instruments over 4 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.(1)

An additional study was performed with Phadia 250 measuring 70 apparently healthy subjects in order to compare the statistical data performed. Results show good agreement.

REFERENCEs