Anti-β2-Glycoprotein I IgA

ORG 521A

96 Tests

Immunometric Enzyme Immunoassay for the quantitative determination of anti-β2-Glycoprotein I-Antibodies of IgA class

Instruction for use
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WARNINGS AND PRECAUTIONS

All reagents of this test kit are strictly intended for in vitro use only. Please adhere strictly to the sequence of pipetting steps provided in this protocol. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. All reagents should be stored refrigerated at 2 - 8 °C in their original container. Do not interchange kit components from different lots. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components beyond their expiration dates. Allow all kit components and specimen to reach room temperature prior to use and mix well. During handling of all kit reagents, controls and serum samples observe the existing legal regulations. The following precautions should be taken handling potentially infectious materials:
- do not eat, drink or smoke in areas where specimens or kit reagents are handled
- do not pipette by mouth
- wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

The test kit contains components of human origin which, when tested by FDA-licensed methods, were found negative for hepatitis B surface antigen and for HIV antibody. No known test can guarantee, however, that products derived from human blood will not be infectious. Handle, therefore, all reagents and human blood derivatives, like plasma or serum samples, as if capable of transmitting infection. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin wash thoroughly with water and soap. The stop solution contains hydrochloric acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.

MATERIALS SUPPLIED

Package size 96 determ.
divisible microplate consisting of 12 modules of 8 wells each, ............ 1 coated with highly purified human β2-Glycoprotein I calibrators with IgA class Anti-β2-Glycoprotein I ................................ 6 vials, 1.5 ml each antibodies in a PBS/BSA matrix containing:
0; 6.3; 12.5; 25; 50; 100 U/ml Anti-β2-Glycoprotein I controls in a PBS/BSA matrix........................... 2 vials, 1.5 ml each (positive and negative), for the respective concentrations see the enclosed package insert sample buffer, yellow, Concentrate ..................................................... 1 vial, 20 ml enzyme conjugate solution, (light red) containing polyclonal .............. 1 vial, 15 ml rabbit anti-h-IgA-IgG; labelled with horseradish peroxidase TMB substrate solution ................................................................. 1 vial, 15 ml stop solution (1 M hydrochloric acid) .............................................. 1 vial, 15 ml buffered wash solution, Concentrate .............................................. 1 vial, 20 ml
CONTROLS
A set of two controls is provided with the kit.

TECHNICAL DATA
Sample material: serum or plasma
Required sample volume: 10 µl of sample to be diluted 1:100 with sample buffer
100 µl prediluted sample per single determination
Total incubation time: 60 minutes at room temperature (20 - 28 °C)
Calibration range: 6.3 - 100 U/ml
Sensitivity: 0.5 U/ml
Storage: refrigerated at 2 - 8 °C
Shelf life: 12 months after manufacturing or until the expiration date printed on the labels
Package size: 96 tests

PRINCIPLE OF THE PROCEDURE
Anti-β2-Glycoprotein I IgA is an indirect solid phase enzyme immunometric assay (ELISA). It is designed for the quantitative measurement of IgA class autoantibodies directed against β2-Glycoprotein I. Preparations of highly purified human β2-Glycoprotein I are coated on high binding, γ-irradiated polystyrene microplates.

The microplates can be divided into 12 modules of 8 wells each or can be used completely for 96 determinations. Each well can be separated from the module ("break-away"). The binding of present autoantibodies, formation of the sandwich complexes and enzymatic colour reaction take place during three different reaction phases:

Phase 1:
Calibrators, controls and prediluted patient samples are pipetted into the wells of the microplate. Any present antibodies bind to the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

Phase 2:
An anti-human-IgA horseradish peroxidase conjugate solution is pipetted into the wells of the microplate to recognize IgA class autoantibodies bound to the immobilized antigens. After a 15 minutes incubation any excessive enzyme conjugate, which is not specifically bound is washed away with wash buffer.

Phase 3:
A chromogenic substrate solution containing TMB (3,3',5,5'-Teramethyl-benzidine) is dispensed into the wells. During 15 minutes of incubation the color of the solutions change into blue. Color development is stopped by adding 1 M hydrochloric acid as stop solution. The solutions color change into yellow. The amount of colour is directly proportional to the concentration of IgA present in the original sample.

To read the optical density a microplate reader with a 450 nm filter is required. Bi-chromatic measurement with a 600-690 nm reference is recommended. The optical density for each calibrator may be graphically plotted against the concentration of IgA and unknowns extrapolated from the curve.
**CLINICAL RELEVANCE**

Anti-β2-Glycoprotein I antibodies are associated with the diseases of the antiphospholipid syndrome, like thrombosis, thrombocytopenia or fetal loss in the context of systemic lupus erythematous.

β2-Glycoprotein I (apolipoprotein H) is a 50 kDa β2-globulin occurring in plasma at a level of 200 µg/ml. It has been found that β2-Glycoprotein I (β2GPI) inhibits the intrinsic coagulation pathway and, therefore, it is involved in the regulation of blood coagulation. β2GPI is associated in vivo with negatively-charged substances, e.g. anionic phospholipids, heparin and lipoproteins. The phospholipid binding region is located on its fifth domain.

Recently, β2-Glycoprotein I has become well-known as a co-factor for anti-Cardiolipin auto-antibodies. Several studies confirmed its indispensable role in proper binding of anti-Cardiolipin antibodies to immobilized Cardiolipin. Detailed investigations about the nature of the Cardiolipin-β2GPI-complex have shown that epitopes on the fifth domain of β2GPI are the real target of "anti-Cardiolipin antibodies" - even in the absence of negatively-charged phosholipids. β2GPI is not only a prerequisite for the binding of anti-Cardiolipin antibodies; it has now been identified as the primary antigen for these antibodies.

Samples from clinical patients with the antiphospholipid syndrome were tested for anti-Cardiolipin and anti-β2GPI antibodies. Good correlations between the anti-Cardiolipin and anti-β2GPI values confirm the statement above.

Autoantibodies against β2-Glycoprotein I are described for various autoimmune diseases. The presence of anti-β2GPI antibodies can be related to the development of arterial and venous thromboses, venous thromboembolism, thrombocytopenia and fetal loss.

Anti-β2-Glycoprotein I antibodies are found in the immunoglobulin classes IgG, IgM and IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune diseases, whereas IgG antibodies will be found in progressive stages of manifested autoimmune disorders. Anti-β2GPI IgG antibody titers correlate well with the clinical status of the patients in thrombosis, thromboembolism and repeated fetal loss, while anti-β2GPI IgM antibodies show a significant association with thrombosis and thrombocytopenia.

Indications for determination of anti-β2-Glycoprotein I antibodies:
- SLE - arterial and venous thromboses
- venous thromboembolism - thrombocytopenia
- fetal loss

**NORMAL VALUES**

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-β2-Glycoprotein I IgA tests:

\[
\text{anti-β2-Glycoprotein I-Ab} \quad \text{IgA [U/ml]}
\]

- normal: < 5
- borderline: 5 - 8
- elevated: > 8

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.

It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-β2-Glycoprotein I.
SPECIFICITY

The microplate is coated with highly purified human β2-Glycoprotein I. The test kit is specific only for autoantibodies against β2-Glycoprotein I.

Endogenous β2-Glycoprotein I and endogenous negatively-charged phospholipids occur in (1:100)-diluted samples at approx. 2 µg/ml and approx. 1 µg/ml, respectively. Influences on the determination of Anti-β2-Glycoprotein I-antibodies have not been observed.

CALIBRATION

Since no international reference preparation for anti-β2-Glycoprotein I autoantibodies is available, the assay system is calibrated in relative arbitrary units.

The calibration is related to the internationally recognized reference sera from E.N. Harris, Louisville. These sera test positive for anti-β2-Glycoprotein I autoantibodies.

REFERENCES

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   β2-Glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway.

3. Lee, N.S. et al.
   β2-Glycoprotein I - Molecular properties of an unusual apolipoprotein, Apolipoprotein H.

   Epitope mapping studies of antiphospholipid antibodies and β2GPI using synthetic peptides.

5. Matsuura, E. et al.
   Molecular studies on phospholipid-binding sites and cryptic epitopes appearing on β2-glycoprotein I structure recognized by anti-cardiolipin antibodies.

6. Koike, T.
   Anticardiolipin Antibodies and β2-Glycoprotein I.
   Clinical Immunology and Immunopathology 1994; Vol 72, No 2: 187 - 192.

   "Anticardiolipin" autoantibodies recognise β2-Glycoprotein I in the absence of phospholipid.

8. Wang, M.-X. et al.
   Epitope specificity of monoclonal anti-β2-Glycoprotein I antibodies derived from patients with the antiphospholipid syndrome.
IgG2 subclass restriction of anti-β2-Glycoprotein I antibodies in autoimmune patients.

Prevalence of β2-glycoprotein I antibody in systemic lupus erythematosus patients with β2-glycoprotein I dependent antiphospholipid antibodies.

11. Martinuzzo, M.E. et al.
Anti β2 glycoprotein I antibodies: detection and association with thrombosis.

Anti-beta2-glycoprotein I antibodies: a marker of antiphospholipid syndrome?

MATERIALS REQUIRED

Equipment
- Microplate reader capable for endpoint measurements at 450 nm
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl

Preparation of reagents
- destilled water
- graduated cylinder for 100 and 1000 ml
- plastic container for storage of the wash solution

Optional
- Multi-Chanel Dispenser
- or repeatable pipet for 100 µl
- data reduction software
SPECIMEN COLLECTION AND PREPARATION

For determination of anti-β2-Glycoprotein I serum or plasma are the preferred sample matrixes. All serum and plasma samples are prediluted 1 : 100 with sample buffer. Therefore 10 µl of sample may be diluted with 1000 µl of sample buffer.

The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation.

Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

PREPARATION AND STORAGE OF REAGENTS

All components of this test kit are supplied in a liquid format and ready to use, except the sample buffer and wash buffer. When stored refrigerated at 2 - 8 °C the components are stable for at least 30 days after opening or until the expiration date printed on the labels.

Remaining modules of the microplate should be stored refrigerated at 2 - 8 °C protected from moisture; store together with desiccant and carefully sealed in the plastic bag.

**Preparation of sample buffer**

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

**Preparation of buffered wash solution**

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

NOTES ON TECHNIQUE

Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

**Pipetting and Sample Handling**

Use a disposable-tip micropipette to dispense sera and plasma samples. Pipet directly to the bottom of the wells. To avoid carryover contamination change the tip between samples.

Patient samples expected to contain high concentrations should be additionally diluted with sample buffer before. Additional dilutions must be considered during calculation.
IMMUNOASSAY PROCEDURE

Do not interchange components of different lots.
All components should be at room temperature before use.
Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 1000 µl of sample buffer in a polystyrene tube. Mix well. Calibrators and controls are ready to use and need not to be diluted.

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and prediluted patient samples in duplicates.

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<tr>
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<th>1</th>
<th>2</th>
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<tr>
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<td>SA</td>
<td>SE</td>
<td>P1</td>
<td>P5</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>SA</td>
<td>SE</td>
<td>P1</td>
<td>P5</td>
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<tr>
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<td>SB</td>
<td>SF</td>
<td>P2</td>
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<tr>
<td>H</td>
<td>SD</td>
<td>C2</td>
<td>P4</td>
<td></td>
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</tbody>
</table>

SA - SF: standards A to F
P1, P2... patient sample 1, 2...
C1: positive control
C2: negative control

2. Pipet 100 µl of calibrators, controls and prediluted patient samples into the wells.
3. Incubate for 30 minutes at room temperature (20 - 28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate solution into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature protected from light.
10. Add 100 µl of stop solution to each well of the modules and leave untouched for 5 minutes.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with reference at 600-650 nm is recommended.

The developed color is stable for at least 30 minutes.
Read optical densities during this time.
CALCULATION OF RESULTS

For anti-β2-Glycoprotein I IgA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log co-ordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

CALCULATION EXAMPLE

The figures below show typical results for anti-β2-Glycoprotein I IgA. These data are intended for illustration only and should not be used to calculate results from another run.

<table>
<thead>
<tr>
<th>No</th>
<th>Position</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean</th>
<th>Conc. 1</th>
<th>Conc. 2</th>
<th>Mean decl.Conc.</th>
<th>CV %</th>
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<tr>
<td>STA</td>
<td>A 1/B 1</td>
<td>0.039</td>
<td>0.037</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>STB</td>
<td>C 1/D 1</td>
<td>0.258</td>
<td>0.248</td>
<td>0.253</td>
<td>6.4</td>
<td>6.0</td>
<td>6.2</td>
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<td>E 1/F 1</td>
<td>0.420</td>
<td>0.425</td>
<td>0.423</td>
<td>12.3</td>
<td>12.5</td>
<td>12.4</td>
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<tr>
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<td>G 1/H 1</td>
<td>0.727</td>
<td>0.746</td>
<td>0.736</td>
<td>25.3</td>
<td>26.2</td>
<td>25.8</td>
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<tr>
<td>STE</td>
<td>A 2/B 2</td>
<td>1.198</td>
<td>1.174</td>
<td>1.186</td>
<td>49.8</td>
<td>48.4</td>
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<td>C 2/D 2</td>
<td>1.947</td>
<td>1.931</td>
<td>1.939</td>
<td>101.1</td>
<td>99.8</td>
<td>100.4</td>
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</table>
ASSAY CHARACTERISTICS

**Sensitivity**
The lower detection limits for anti-β2-Glycoprotein I IgA were determined at 0.5 U/ml.

**Parallelism**
In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the anti-β2-Glycoprotein I IgA kit. The assay shows linearity over the full measuring range.

INCUBATION SCHEME

1. Pipet **100 µl** calibrator, control or diluted patient sample
   - Incubate for **30 minutes** at room temperature
   - Discard the contents of the wells and wash 3 times with **300 µl** wash solution

2. Pipet **100 µl** enzyme conjugate
   - Incubate for **15 minutes** at room temperature
   - Discard the contents of the wells and wash 3 times with **300 µl** wash solution

3. Pipet **100 µl** substrate solution
   - Incubate for **15 minutes** at room temperature

4. Add **100 µl** stop solution
   - Leave untouched for **5 minutes**
   - Read at **450 nm**