Phadia 100

EliA™

Not for use in the USA

β2-Glycoprotein I IgA

FLUOROENZYMEIMMUNOASSAY FOR ANTI β2-GLYCOPROTEIN I ANTIBODIES

FOR IN VITRO DIAGNOSTIC USE

DIRECTIONS FOR USE

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 β2-Glycoprotein I IgA is intended for the in vitro quantitative measurement of IgA antibodies directed to β2-Glycoprotein I in human 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 β2-Glycoprotein I IgA uses the EliA IgA method on the instrument Phadia 100.

SUMMARY AND EXPLANATION OF THE TEST

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 antiphospholipid antibodies. The criteria for classification of the APS have been revised in 2004 in Sydney[1]. Besides the clinical criteria, three different laboratory tests are listed: lupus anticoagulant, anticardiolipin antibodies (IgG and IgM) and anti-β2-Glycoprotein I antibodies (IgG and IgM). The latter was not included in the former Sapporo criteria. However, by majority, the Sydney committee agreed that they are an independent risk factor for thrombosis and pregnancy complications[1]. The agreement achieved in Sydney was that so far that it is premature to implement IgA Anti-β2-Glycoprotein I antibodies into the new criteria. Anti-β2-Glycoprotein I antibodies of the IgA isotype are not part of the classification criteria as data did not reach sufficient evidence level to support the presence as independent risk factor.

For APS diagnosis, β2-Glycoprotein I antibody tests show higher specificity than anticardiolipin assays[2]. In 3-10% of APS patients, β2-Glycoprotein I antibodies may be the only positive test[3]. The association of β2-Glycoprotein I antibodies with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for anticardiolipin antibodies implies that the inclusion of β2-Glycoprotein I antibodies may also help clarify this type of pregnancy morbidity[4]. Outside the context of clinical studies, testing for β2-Glycoprotein I antibodies can be helpful for APS diagnosis, particularly when anticardiolipin antibodies and lupus anticoagulant are negative and APS is strongly suspected[5].

PRINCIPLES OF THE PROCEDURE

The EliA β2-Glycoprotein I IgA Wells are coated with human β2-Glycoprotein I antigen. If present in the patient's specimen, antibodies to β2-Glycoprotein I bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgA antibodies (EliA IgA 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 IgA 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 100 and the EliA IgG/IgM/IgA Negative Control 100 are required to carry out an EliA β2-Glycoprotein I IgA Test.

The EliA β2-Glycoprotein I IgA Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA β2-Glycoprotein I IgA Test-Specific Reagents

EliA β2-Glycoprotein I IgA Well (Art. No. 14-5531-01)

β2-Glycoprotein I IgA Well; short name: Ab2	coated with human β2-Glycoprotein I antigen	2 carriers (12 wells each); sufficient for 24 determinations	ready for use; store dry at 2-8 °C until expiration date

EliA APS Positive Control 100 (Art. No 83-1054-01)

Human monoclonal antibodies in Tris buffer; symbol: pos	Multiparameter control containing IgG/IgM/IgA antibodies to cardiolipin and β2-Glycoprotein I	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 100 (Art. No 83-1042-01)

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 IgG/IgM/IgA Negative Control 100 is prepared from selected pooled human sera.

EliA Method-Specific Reagents (Phadia 100)

EliA Sample Diluent (Art. No 83-1003-01)

Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095 %)	6 vials (9 ml each); sufficient for 6 x 30 dilutions (instrument 1:100 dilution)	ready for use; store at 2-8 °C until expiration date

EliA IgA Conjugate (Art. No 83-1014-01)

IgA Conjugate (blue colored); ß-Galactosidase anti-IgA (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-A	6 vials (4.8 ml each); sufficient for 6 x 48 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE

EliA IgA Conjugate (Art. No 83-1012-01)

IgA Conjugate (blue colored);ß-Galactosidase anti-IgA (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-A	2 vials (4.8 ml each); sufficient for 2 x 48 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE

EliA IgA Calibrators (Art. No 83-1013-01)

human IgA (0, 0.3, 1.5, 5, 15, 80 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CAL-0, CAL-0.3, CAL-1.5, CAL-5, CAL-15, CAL-80	6 single-use vials (0.3 ml each);sufficient for one calibration curve	ready for use; store at 2-8 °C until expiration date

Manufactured from human sera.

EliA IgA Curve Control (Art. No 83-1010-01)

human IgA (5 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CC-1	6 single-use vials (0.3 ml each);sufficient for 6 runs	ready for use; store at 2-8 °C until expiration date

Manufactured from human sera.

EliA IgA Calibrator Well (Art. No 14-5516-01)

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

EliA Dummy Well (Art. No 14-5510-01)

Dummy Well required by the Phadia 100 System for empty run positions	4 carriers (12 wells each); sufficient for 48 positions	ready for use; store dry at 2-8 °C until expiration date

Phadia 100 General Reagents

Development Solution (Art. No. 10-9478-01)

Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010 % preservative**	6 vials (6 ml each); reagents for 6 x 48 determinations	ready for use; store at 2-8 °C until expiration date. DO NOT FREEZE

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

Stop Solution 4 % Sodium Carbonate	6 bottles (65 ml each); *reagents for 6 x 240 determinations	ready for use; store at 2-8 °C until expiration date

* Due to different ImmunoCAP and EliA assay processes, a high residual volume is to be expected.
** 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. 220-239-6] (3:1).

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

For information see separate Washing Solution package insert.

The expiration date for each of the complete packages is stated on the outer label. However, each component is stable until the date stated on the respective vial label.

Material not provided: purified water, graduated cylinder

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 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 (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 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 100 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 100 User's Guide/Reference Manual.

INSTRUMENT

The Phadia 100 Instrument processes all steps of the test and prints results automatically after the test is completed. For further information regarding test set-up, instrumentation and software etc. see Phadia 100 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.
CLSI-Document H18-A3 recommends the following storage conditions for samples:

- Separated serum/plasma should remain at room temperature for no longer than eight hours.
- If assays will not be completed within eight hours, serum/plasma should be refrigerated (2 to 8°C).
- If assays are not completed within 48 hours, or the separated serum/plasma will be stored beyond 48 hours, serum/plasma should be frozen at or below -20°C. Avoid repeated freezing and thawing.

Sample Dilution

Samples must be diluted with EliA Sample Diluent. A 1:10 dilution of the samples is required for the EliA β2-Glycoprotein I IgA Test. Samples can be diluted manually, but instrument dilution is recommended and is a default setting in Phadia 100 Instrument Software.

PROCEDURE

Handling of EliA β2-Glycoprotein I IgA Well

Prior to opening the foil bag, equilibrate to room temperature. For stability reasons the carriers have to be put back in the desiccant-containing foil bag directly after dispensing the wells. Because it is important to store the wells in dry conditions at 2-8 °C, the bag must be properly resealed. Shelf-life after first opening: 9 months, if not limited by expiry date stated on the carrier and foil bag.

Lot-specific code of EliA β2-Glycoprotein I IgA Well

Make sure to enter the lot-specific code of the EliA β2-Glycoprotein I IgA Well. This code is stated on the carrier and foil bag as Code, and it is encoded within the barcode of the foil bag. Preferably use a barcode reader.

Lot-specific code of EliA IgA Calibrator Well

Make sure to enter the lot-specific code of the EliA IgA Calibrator Well. This code is stated on the carrier and foil bag as Code, and it is encoded within the barcode of the foil bag. Preferably use a barcode reader.

Lot specific code of EliA IgA Conjugate
Make sure to enter the lot-specific Calibration Code of the IgA Conjugate given on the box and the vial as CalCode, and encoded within the barcode of the IgA Conjugate. Preferably use a barcode reader to enter the Calibration Code.

Volumes per determination
Reagent volumes per determination

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

Sample volumes per determination

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

For tube-specific dead volumes see Phadia 100 User's Guide/Reference Manual.

Reagent volumes per run

Washing Solution	1 l*
Rinse Solution	1 l*

* 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.
- Total time is 2.5 hours for one test run processing 48 wells.
- Incubations are automatically performed at 37 °C (98.6 °F) by Phadia 100.

CALIBRATION AND REFERENCE MATERIAL

The calibration curve is obtained with EliA IgA Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA IgA Curve Control (run in duplicate).
The IgA 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 IgA Conjugate is introduced or
- when the EliA IgA Curve Control is outside the specified limits (defined in Phadia 100 Instrument Software).

There are no international standards for β2-Glycoprotein I antibodies. Results are given in arbitrary EliA Units/ml.

QUALITY CONTROL

Record Keeping
It is good laboratory practice to record the lot numbers of the components used, the dates when they were first opened and remaining volumes.

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 100 measures specific IgA concentrations in µg/l. By using a conversion factor given by the lot-specific code of the EliA β2-Glycoprotein I IgA Well, the results are automatically converted to U/ml.

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

Test	Unit	negative	equivocal	positive
EliA β2-Glycoprotein I IgA	U/ml	< 7	7 - 10	> 10

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 the β2-Glycoprotein I antibodies covered by the EliA β2-Glycoprotein I IgA test is below 3 %[6]. Expected values may vary depending on the population tested.

Results Obtained for Healthy Subjects
The frequency distribution for β2-Glycoprotein I IgA antibodies was investigated on the instrument Phadia 250 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)]

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA β2-Glycoprotein I IgA	U/ml	400	1.8	4.3	8.7

A comparison study between Phadia 100 and Phadia 250 was performed with 36 patient samples to assess the analytical performance of both systems. Results show good agreement.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA β2-Glycoprotein I IgA is from 0.2 to ≥ 183 U/ml. No hook effects could be observed for concentrations up to 12 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 µg/l to 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 β2-Glycoprotein I IgA Test permits the determination of IgA antibodies directed against the β2-Glycoprotein I 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 84 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.(1)

Test	Sample	Unit	Mean value	Coefficients of variation (%)	
				Intra-Run	Inter-Run
EliA β2-Glycoprotein I IgA	1	U/ml	9.7	2.7	3.0
	2	U/ml	25.7	2.9	3.4
	3	U/ml	135.9	3.3	5.3

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.

(1) Studies performed at Phadia GmbH, Freiburg, Germany

REFERENCES

1. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. J Thromb Haemost 2006; 4:295-306
2. Obermoser G, Bitterlich W, Kunz F, Sepp NT. Clinical significance of anticardiolipin and anti-beta(2)- glycoprotein I antibodies. Int.Arch.Allergy Immunol. 2004; 135 (2):148-153
3. Lee EY, Lee CK, Lee TH, et al. Does the anti-beta2-glycoprotein I antibody provide additional information in patients with thrombosis? Thromb Res 2003; 2:1077-1081
4. Faden D, Tincani A, Tanz P, et al. Anti-beta2 glycoprotein I antibodies in a general obstetric population: preliminary results on teh prevalence and correlation with pregnancy outcome. Anti-beta2 glycoprotein I antibodiesare associated with some obstetrical complications, mainly preeclampsia-eclampsia. Eur J Obstet Gynecol Reprod Biol 1997; 73:37-42
5. Villalta D. Antiphospholipid syndrome update: from classification to treatment. CLI-online 2007; 7:8-11
6. Bruce IN, Clark Soloninka CA, Spitzer KA, Gladman DD, Urowitz MB, Laskin CA. Prevalence of antibodies to beta(2)-glycoprotein I in systemic lupus erythematosus and their association with antiphospholipid antibody syndrome criteria: A single center study and literature review. J.Rheumatol. 2000; 27 (12):2833-2837

LOT	Batch code
	Biological Risk
	Store at 2-8°C/35-46°F
	Expiration date
IVD	For *in vitro* diagnostic use
Σ	Contains x determinations
	Read Directions for Use
	Manufactured by
	Do not reuse in a second run

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Issued June 2013