EliA™

M2 FLUOROENZYMEIMMUNOASSAY FOR M2 ANTIBODIES

FOR IN VITRO DIAGNOSTIC USE   **DIRECTIONS FOR USE**

Not for use in the USA

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 M2 is intended for the in vitro quantitative measurement of IgG antibodies directed to M2 in human serum and plasma as an aid in the clinical diagnosis of primary biliary cirrhosis. EliA M2 uses the EliA IgG method on the instrument Phadia 100.

SUMMARY AND EXPLANATION OF THE TEST
Primary biliary cirrhosis (PBC) is a chronic, cholestatic liver disease which affects mainly middle-aged women. It starts with an inflammatory process of the small and middle-sized interlobular bile ducts leading first to a proliferation and then to a loss of bile ducts, to portal inflammation and in late stages to liver cirrhosis[1, 2].

PBC occurs all over the world but with varying incidence, ranging from 0.7-49 per million per year. In most recent studies the point prevalence was estimated to range from 6.7 to 402 per million[3].

Typical clinical features of PBC are fatigue, pruritus and Sicca-syndrome[1]. However, nowadays at diagnosis, the majority of patients are asymptomatic and present for other reasons, e.g. for workup of elevated serum levels of AP or cholesterol. A diagnosis of PBC is made "with confidence" when biochemical markers of cholestasis, particularly alkaline phosphatase, are elevated persistently for more than 6 months in the presence of antibodies against mitochondria and in the absence of an alternative explanation[1].

PBC-related antibodies against mitochondria react with subunits of the 2-oxoacid-dehydrogenase complex (2-OADC) and, in most cases, recognize the E2-subunit of pyruvate dehydrogenase (PDH-E2)[4]. Individuals who are positive for these so-called anti-M2 antibodies, even if they have no signs of cholestasis and/or liver inflammation, are very likely to develop PBC. Anti-M2 antibodies are present in about 95% of PBC-patients.[1, 2]

PRINCIPLES OF THE PROCEDURE
The EliA M2 Wells are coated with native pyruvate dehydrogenase complex from mitochondria and recombinant M2-antigen. If present in the patient's specimen, antibodies to M2 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
EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA M2 Positive Control 100 and the EliA IgG/IgM/IgA Negative Control 100 are required to carry out an EliA M2 Test.

The EliA M2 Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA M2 Test-Specific Reagents
EliA M2 Well (Art. No. 14-5649-01)

M2 Well; short name: m2G	coated with native pyruvate dehydrogenase complex from mitochondria and recombinant M2-antigen	4 carriers (12 wells each); sufficient for 48 determinations	ready for use; store dry at 2-8 °C until expiration date

EliA M2 Positive Control 100 (Art. No 83-1103-01)

Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos	Control containing IgG antibodies to M2	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 M2 Positive Control 100 is prepared from selected pooled human sera.

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	ready for use; store at 2-8 °C until expiration date

EliA IgG Conjugate (Art. No 83-1002-01)

IgG Conjugate (blue colored); ß-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: El-G	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 IgG Conjugate (Art. No 83-1005-01)

IgG Conjugate (blue colored); ß-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: El-G	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 IgG Calibrators (Art. No 83-1000-01)

human IgG (0, 4, 10, 20, 100, 600 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: Cal-0, Cal-4, Cal-10, Cal-20, Cal-100, Cal-600	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 IgG Curve Control (Art. No 83-1001-01)

human IgG (20 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 IgG Calibrator Well (Art. No 14-5509-01)

IgG Calibrator Well coated with mouse monoclonal antibodies; short name: Gcal	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

* 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).
** Due to different ImmunoCAP and EliA assay processes, a high residual volume is to be expected.

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-A4 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 1:100 dilution of the samples is required for the EliA M2 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 M2 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 M2 Well
Make sure to enter the lot-specific code of the EliA M2 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 IgG Calibrator Well
Make sure to enter the lot-specific code of the EliA IgG 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 IgG Conjugate
Make sure to enter the lot-specific Calibration Code of the IgG Conjugate given on the box and the vial as CalCode, and encoded within the barcode of the IgG Conjugate. Preferably use a barcode reader to enter the Calibration Code.

Volumes per determination
Reagent volumes per determination

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

Sample volumes per determination

Manual dilution:	90 µl of diluted sample
Instrument dilution (1:100):	22 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

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

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).

EliA M2 is calibrated against the non-WHO Reference Material Primary Biliary Cirrhosis Serum, Human NIBSC code 67/183. Results are given in International Units (IU/ml).

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 100 Instrument Software).

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 IgG concentrations in µg/l. By using a conversion factor given by the lot-specific code of the EliA M2 Well, the results are automatically converted to IU/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 M2	IU/ml	<4	4−6	>6

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. Anti-M2 antibodies are highly specific and usually do not occur in healthy blood donors. However, they may predict the future development of PBC in seropositive cases at early stages of the diseases where no symptoms suggestive of cholestatic disease are detectable.[4] Expected values may vary depending on the population tested.

Results Obtained for Healthy Subjects

The frequency distribution for M2 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.

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA M2	IU/ml	400	0.8	1.5	3.4

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA M2 is from 0.5 to ≥ 220 IU/ml.

No hook effects could be observed for concentrations up to 7 fold above the measuring ranges.

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 IU/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 M2 Test permits the determination of IgG antibodies directed against the M2 antigen as described in section "Reagents".

Precision

To determine the precision of the assay, the variability was assessed in a study with 7 runs by examining the samples in 84 replicates and on 3 instruments over 7 days with a calibration curve included into each run.

Test	Sample	Unit	Mean value	Coefficients of variation (%)	
				Intra-Run	Inter-Run
EliA M2	1	IU/ml	4.9	5.2	2.5
	2	IU/ml	29.1	5.9	1.8
	3	IU/ml	174.8	5.3	5.6

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

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

🏭 **Phadia AB**
Rapsgatan 7P
P.O. Box 6460
751 37 Uppsala
Sweden
Tel: +46-18-16 50 60
Fax: +46-18-14 03 58
Autoimmunity@phadia.com
www.phadia.com

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