Phadia 250

EliA™ SmD^P

Not for use in the USA

FLUOROENZYMEIMMUNOASSAY FOR ANTI Sm ANTIBODIES
DIRECTIONS FOR USE

FOR IN VITRO DIAGNOSTIC 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 SmD^P is intended for the in vitro quantitative measurement of IgG antibodies directed to Sm in human serum and plasma as an aid in the clinical diagnosis of systemic lupus erythematosus (SLE). EliA SmD^P uses the EliA IgG method on the instrument Phadia 250.

SUMMARY AND EXPLANATION OF THE TEST

The determination of antinuclear antibodies (ANA) is of central importance for the clinical diagnosis of systemic lupus erythematosus (SLE). Sm antibodies, and particularly those against the SmD component, offer a highly specific, but comparatively insensitive, clinical marker for SLE. Indeed, their presence constitutes one of the revised ACR criteria for diagnosis, even though their overall prevalence ranges from 20 % to 30 % in SLE.[1,2] Anti-Sm antibodies react with the proteins BB' and D. However, tests which include the antigens BB' fail to differentiate patients with SLE from those with other autoimmune diseases. Only SmD is considered the most SLE-specific antigen.[3] The ability of SmD-based antibody tests to differentiate between SLE and other autoimmune diseases can even be improved by using an SmD_3 peptide as antigen.[4]

PRINCIPLES OF THE PROCEDURE

The EliA SmD^P Wells are coated with a synthetic SmD_3 peptide. If present in the patient's specimen, antibodies to the SmD_3 peptide 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

The EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA ANA Positive Control 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA SmD^P Test.
The EliA SmD^P Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA SmD^P Test-Specific Reagents

EliA SmD^P Well (Art. No. 14-5624-01)

SmD^P Well; short name: smd	coated with a synthetic SmD_3 peptide	4 carriers (12 wells each); sufficient for 48 determinations	ready for use; store dry at 2-8 °C until expiration date

EliA ANA Positive Control 250 (Art. No 83-1033-01)

Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos	Multiparameter control containing IgG antibodies to dsDNA, RNP, Sm, Ro, La, Scl-70, CENP and Jo-1	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 ANA Positive Control 250 is prepared from selected pooled human sera.

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 IgG/IgM/IgA Negative Control 250 is prepared from selected pooled human sera.

EliA Method-Specific Reagents (Phadia 250)

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); ß-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); ß-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.

EliA IgG Curve Control Strips (Art. No 83-1016-01)

human IgG (20 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 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

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 DO NOT FREEZE

Development Solution (Art. No. 10-9441-01)

Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010 % preservative*	6 bottles (11 ml each); sufficient for 6 x >110 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE

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 2-32 °C until expiration date

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

Stop Solution 4 % Sodium Carbonate	6 bottles (65 ml each); sufficient for 6 x >292 determinations	ready for use; store at 2-32 °C until expiration date

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

MicroWell ™ plates with 96 wells, 0.5 ml each; Polypropylene	100 plates per package; sufficient for 100 x 96 samples	ready for use DO NOT REUSE

* 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.

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 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.
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 1:50 dilution of the samples is required for the EliA SmD^P Test. Samples can be diluted manually, but instrument dilution is recommended.

PROCEDURE

Handling of EliA SmD^P 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.

Lot specific barcode

Use the built-in barcode reader to enter the lot specific information of EliA SmD^P Well, EliA IgG Calibrator Well and EliA IgG 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.
- **EliA Sample Diluent**
  Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.

- **EliA Conjugate**
  Single use reagent, open vials must not be stored.
- **Development Solution**
  Can be stored on-board for a total of 40h at room temperature. Can be used 5 times during shelf life and be stored at room temperature for 8 hours on each occasion. Re-cap bottles every night. During weekends or longer interval between instrument usage it is recommended to store bottles at 2-8°C.
- **Stop Solution**
  Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.
- **Washing Solution**
  Prepared solution can be stored on-board for 7 days at room temperature. Discard every seventh day and perform weekly maintenance according to instrument user manual.

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 (1:50):	20 µl of non diluted sample

For tube-specific dead volumes see Phadia 250 User's Guide/Reference Manual.

Reagent volumes per 200 determinations

Washing Solution	5-7 l*
Rinse Solution	5-6 l*

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

Procedural comments

- From one sample diluted by the instrument (1:50), up to 5 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).
- Software ≥ 2.20: If you want to perform more than one test per patient you can also use the following predefined test panels:

Name	Description	EliA tests inlcuded
pana	Connective tissue disease	dsDNA, U1RNP, RNP70, SmD^P, Ro, La, Scl-70, CENP, Jo-1
pena	ENA	U1RNP, RNP70, SmD^P, Ro, La, Scl-70, CENP, Jo-1
psle	Systemic lupus erythematosus	dsDNA, SmD^P, Ro, La
pspe	Speckled pattern	U1RNP, SmD^P, Ro, La

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).

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 250 Instrument Software).

There are no international standards for Sm 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 SmD^P 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.

Test	Unit	negative	equivocal	positive
EliA SmD^P	EliA 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.
In rare cases, interference due to extremely high titers of antibodies to streptavidin can occur.

EXPECTED VALUES

Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for Sm antibodies covered by the EliA SmD^P test is below 1 %. Expected values may vary depending on the population tested.[1,2,4,5, 6]

Results Obtained for Healthy Subjects

The frequency distribution for Sm 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 SmD^P	EliA U/ml	400	1.4	3.0	6.7

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA SmD^P is from 0.6 to ≥ 480 EliA U/ml. No hook effects could be observed for concentrations up to 8 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 EliA 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 SmD^P Test permits the determination of IgG antibodies directed against the Sm antigen as described in section “Reagents”.

Precision

To determine the precision of the assay, the variability was assessed in studies with 7 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.

Test	Sample	Unit	Mean value	Coefficients of variation (%)	
				Intra-Run	Inter-Run
EliA SmD^P	1	EliA U/ml	7.7	6.5	1.6
	2	EliA U/ml	17.5	5.3	3.4
	3	EliA U/ml	377.7	4.0	2.6

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

1. Peng SL, Craft JE (1996) Spliceosomal snRNPs autoantibodies. In: Peter JB, Shoenfeld Y (eds), Autoantibodies, pp 774-782, Elsevier, Amsterdam
2. Tan EM (1999) Autoantibodies in Diagnosis and in Identifying Autoantigens. Immunologist 7, 85-92
3. Smolen JS, Hassfeld W, Graninger W, Steiner G (1990) Antibodies to antinuclear subsets in systemic lupus erythematosus and rheumatoid arthritis. Clin Exp Rheumatol 8 (Suppl 5), 41-44
4. Mahler M, Stinton LM, Fritzler MJ (2005) Improved serological differentiation between systemic lupus erythematosus and mixed connective tissue disease by use of an SmD3 peptide-based immunoassay. Clin Diagn Lab Immunol 12, 107-113
5. Smeenk RJT, Berden JHM, Swaak AJG (1996) dsDNA Autoantibodies. In: Peter JB, Shoenfeld Y (eds), Autoantibodies, pp 227-236, Elsevier, Amsterdam
6. van den Hoogen FHJ, van de Putte LBA (1996) Anti-U1snRNP antibodies and clinical associations. In: van Venrooij WJ, Maini RN (eds), Manual of Biological Markers of Disease, pp C3.1, 1-8, Kluwer Academic Publishers, Dordrech

LOT	Batch code	Σ	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		

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