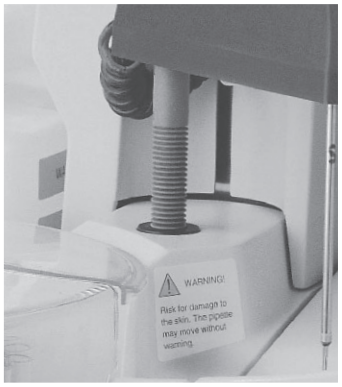


Antiphospholipid Syndrome

Excellence in Autoimmunity

EliA™ Cardiolipin and EliA™ β2-Glycoprotein I – Fully Automated Testing for Antiphospholipid Syndrome (APS)

Testing for APS according to classification criteria

- determination of anti-β2-glycoprotein I IgG and IgM antibodies
- determination of anticardiolipin IgG and IgM antibodies
- use of consensus cut-off for anticardiolipin antibodies

High clinical relevance

- excellent diagnostic performance in routine use
- high clinical sensitivity and specificity
- very good performance with sera from quality assessment schemes

High technical performance

- low variances and high reproducibility for consistent results
- high lot-to-lot consistency due to validated GMP production procedures

Efficiency also in calibration

- one curve for all EliA IgG tests
- one curve for all EliA IgM tests
- parameter-independent calibration makes small runs cost-effective
- calibrated against international standards

Easy handling

- seruma as well as plasma can be used
- automated sample dilution

The EliA™ System

Time for the essentials

- completely automated (true walk-away, overnight runs)
- easy instrument management by Phadia Information Data Manager (IDM) software
- barcode-reader
- protocols, QC and raw data easily accessible
- optional host link
- detailed QC management
- integrated stock management system on the Phadia 250

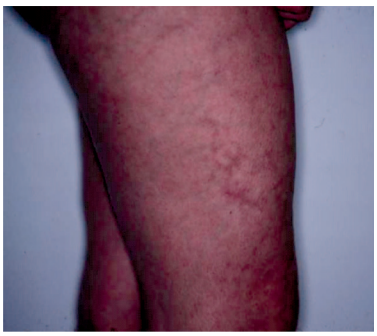
Cost efficient and flexible

- autoimmunity and allergy on the same instrument, in the same run
- different autoimmune tests in the same run
- no batching of samples necessary – small runs can be handled cost-effectively
- once-monthly calibration – curve control each run
- from one to five Phadia instruments may be linked into one IDM computer

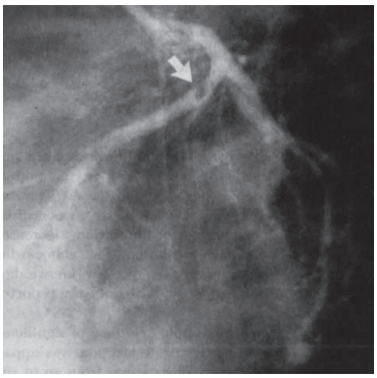
A boost in service for your laboratory and your clinicians

- sample-result turnaround the same day
- STAT function on Phadia 250 for immediate testing of emergency samples
- overnight runs possible
- detailed documentation of results (patient or requester specific)
- Phadia 100 – up to 46 determinations in less than 2.5 hours
- Phadia 250 – fully automated random access with up to 350 determinations per shift
- multiple methods in one run
- positive identification and traceability of samples and reagents on Phadia 250

Antiphospholipid Syndrome



Picture 1



Picture 2

Livedo reticularis (picture 1) and coronary artery thrombosis (picture 2) as examples for manifestations in APS

The antiphospholipid syndrome (APS), also called Hughes syndrome, is a systemic autoimmune disorder characterized by a combination of arterial and/or venous thrombosis, obstetric morbidity (mainly recurrent fetal losses), often accompanied by a mild-to-moderate thrombocytopenia, and elevated titers of antiphospholipid antibodies, namely lupus anticoagulant, anticardiolipin antibodies and/or antibodies to β2-glycoprotein I.
According to the latest classification criteria for APS – the Sydney Classification – the diagnosis of APS requires at least one clinical and one laboratory criterion. The serological proof of antiphospholipid antibodies (IgG and IgM isotypes) is one of two possible laboratory criteria necessary for diagnosis, whereas β2-glycoprotein I (β2GPI) and cardiolipin have been identified as the main antigenic factors in APS.

The Antigens

Phospholipids are major components of the membranes of all living cells and of their organelles. Anticardiolipin antibodies bind stronger if the proteinogenic cofactor β2-glycoprotein I is additionally present, and there is evidence that anti-β2GPI reactivity is related to the pathogenic potential of antiphospholipid antibodies. Moreover, some of the antiphospholipid antibodies show reactivity against this cofactor even in the absence of cardiolipin if attached in high density to solid surfaces.
EliA Cardiolipin assays use highly purified bovine cardiolipin and bovine β2-glycoprotein I as cofactor, following the international standardisation guidelines. EliA β2-Glycoprotein I assays use human purified β2-glycoprotein I as antigen.

High Clinical Relevance

Clinically evaluated with a large serum panel

All four EliA APS tests were validated in a comprehensive study design. Two APS serum panels from two different centers (panel 1 and panel 2) representing 51 and 50 APS patients, respectively, and sera from 50 patients with systemic lupus erythematosus (SLE) were tested. In addition, samples from 301 patients suffering from various other diseases have been analyzed (table 1).
It has been shown, that antiphospholipid antibodies occur in about 30% of SLE patients. In general, such antibodies indicate a risk for thrombotic events. On the other hand affected patients may still lack clinical evidence of antiphospholipid syndrome. Therefore, the results obtained in this group were neither classified as false positive nor as false negative.
In the revised classification criteria from Sydney, a clinical cut-off of > 40 GPL-, MPL-U/ml was defined for anticardiolipin testing (Miyakis S et al, J Thromb Haemost 2006;4:295-306). The EliA Cardiolipin assays are the first commercially available tests applying this consensus cut-off to make the diagnostic interpretation easier.

Clinical background	No. of samples	EliA Cardiolipin IgG	EliA Cardiolipin IgM	EliA β2-Glycoprotein I IgG	EliA β2-Glycoprotein I IgM
		No. of weak pos. / pos. (Total positive in %)	No. of weak pos. / pos. (Total positive in %)	No. of positive (in %)	No. of positive (in %)
APS Panel 1	51	7 / 21 (54.9 %)	10 / 1 (21.6 %)	24 (47.1 %)	11 (21.6 %)
APS Panel 2	50	15 / 22 (74.0 %)	13 / 6 (38.0 %)	35 (70.0 %)	13 (26.0 %)
SLE	50	11 / 6 (34.0 %)	8 / 3 (22.0 %)	13 (26.0 %)	9 (18.0 %)
CTD (ex. SLE)	50	8 / 0 (16.0 %)	3 / 1 (8.0 %)	1 (2.0 %)	5 (4.0 %)
Syphilis	14	1 / 0 (7.1 %)	1 / 0 (7.1 %)	0 (0 %)	0 (0 %)
Viral infections	111	2 / 2 (3.6 %)	11 / 0 (9.9 %)	8 (7.2 %)	3 (2.7 %)
Non-viral infections	24	0 / 1 (4.2 %)	1 / 0 (4.2 %)	1 (4.2 %)	0 (0 %)
Rheumatoid arthritis	40	2 / 0 (5.0 %)	5 / 0 (12.5 %)	0 (0 %)	2 (5.0 %)
Autoimmune thyroid diseases	30	2 / 0 (6.7 %)	0 / 0 (0 %)	2 (6.7 %)	0 (0 %)
Tumor	10	0 / 0 (0 %)	0 / 1 (10.0 %)	0 (0 %)	0 (0 %)
Other	22	0 / 0 (0 %)	0 / 1 (4.6 %)	0 (0 %)	1 (4.5 %)

Table 1: *Performance of EliA Cardiolipin (weak pos.: 10-40 GPL-, MPL-U/ml; pos.: > 40 GPL-, MPL-U/ml) and EliA β2-Glycoprotein I (pos: >10 EliA U/ml)*

Results between 10 and 40 GPL-, MPL-U/ml are classified as low positive. Cardiolipin IgG antibodies in this area were particularly found in SLE (n = 11; 22%) and other connective tissue diseases (n = 8; 16%). The low positive area is not comparable to the equivocal range in other parameters but has a clearly higher relevance. Patients with persistent positivity should be monitored regularly. Without clinical findings, a persistent *high* positive result indicates an increased risk for thrombosis or pregnancy complications and possible diagnosis of APS in the future.

EliA™ Cardiolipin is the first commercial test using the "clinical" cut-off of 40 U/ml

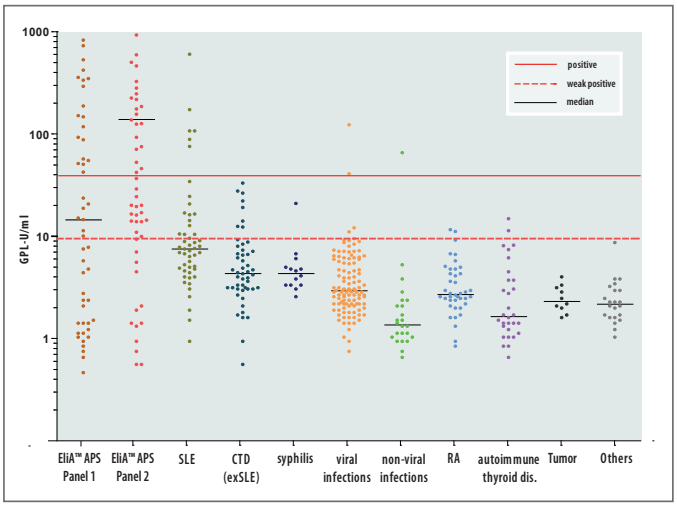


Figure 1: *EliA Cardiolipin IgG in different clinical groups*

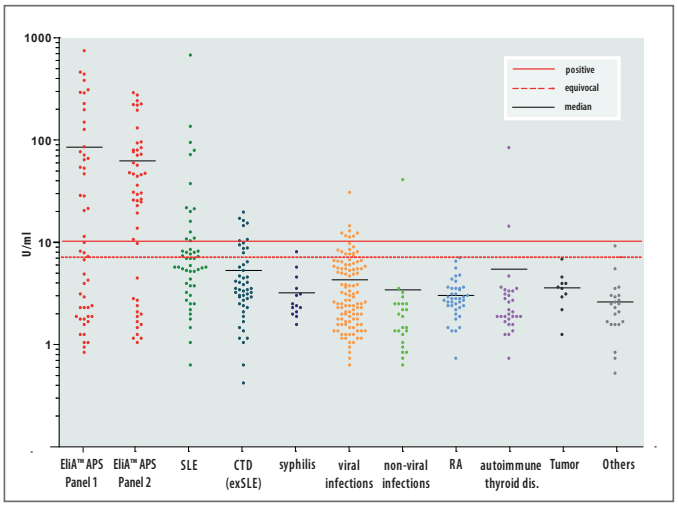


Figure 2: *EliA β2-Glycoprotein I IgG in different clinical groups*

The presence of β2GPI antibodies was included as criterion in the revised classification criteria for APS (Sydney, 2004) which acknowledges their clinical relevance. Due to the lack of international standardization, EliA β2-Glycoprotein I results are given in arbitrary EliA Units. The cut-off is defined at 10 EliA U/ml with an equivocal range from 7 to 10 EliA U/ml.

EliA™ β2-Glycoprotein I – a valuable diagnostic help

Using EliA Cardiolipin and EliA β2-Glycoprotein I tests, the differentiation of APS from diseases which are potentially positive for antiphospholipid antibodies is excellent (see table 1 and figures 1 and 2).

Clinical Performance in a Comparative Study

Excellent clinical sensitivity and specificity

The evaluation of the clinical performance of anticardiolipin or anti-β2GPI tests is a challenge because there is no gold standard for the testing of cardiolipin or β2GPI antibodies. A direct comparison to an existing test does not give an objective impression of the performance of an assay. In a clinical evaluation it is difficult to define APS patients properly as the diagnosis of APS is directly dependent on the results of the tests which shall be evaluated. This bias makes a clear differentiation between correct and false positive results impossible.

In order to minimize this bias, the performance of the new EliA APS tests was evaluated in a study where each assay was compared to three commercial tests, using two different APS panels. Both panels of 50 and 51 APS patients (as mentioned above) were defined by different APS specialists. The resulting test performance is shown in figure 3 – 6 (orange: Sensitivity in 51 APS patients, panel 1; red: Sensitivity in 50 APS patients, panel 2; blue: Specificity in 301 disease controls) .

The data demonstrates the excellent ability of EliA Cardiolipin and EliA β2-Glycoprotein I assays to assist in the diagnosis of APS.

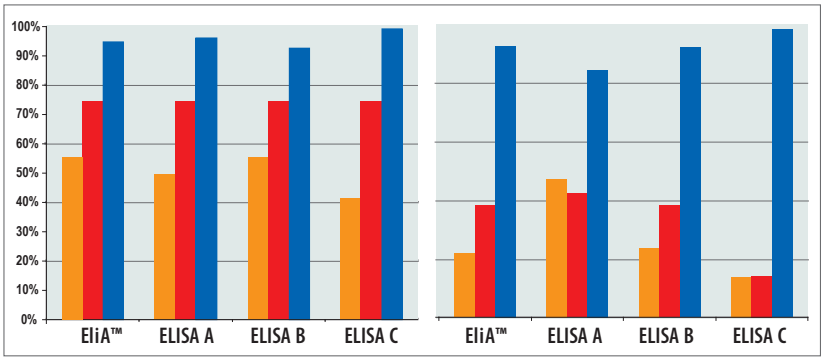


Figure 3: *Cardiolipin IgG*

Figure 4: *Cardiolipin IgM*

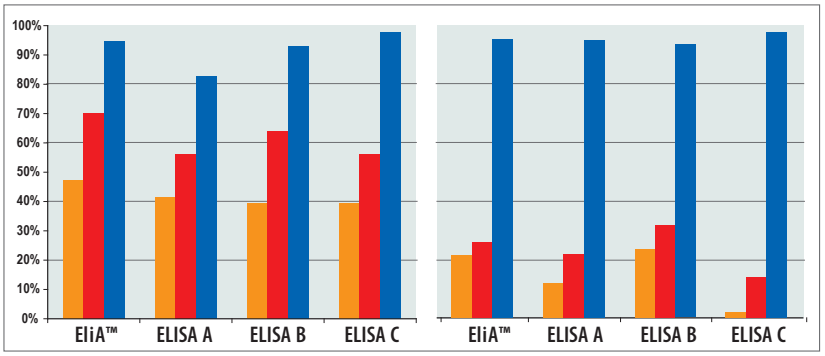


Figure 5: *β2-glycoprotein I IgG*

Figure 6: *β2-glycoprotein I IgM*

High Level of Standardization

Calibrated against international standards

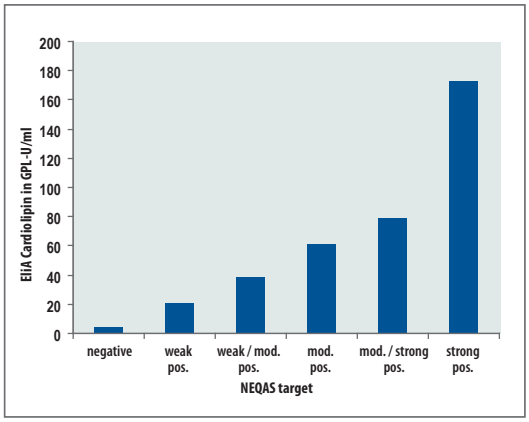
EliA Cardiolipin is calibrated against the internationally accepted Harris sera (Harris EN et al, Clin Exp Immunol 1987;68:215-22) and results are expressed in GPL-U/ml (correlation to Harris set IgG: r = 0.987) and MPL-U/ml (correlation to Harris set IgM: r = 0.953).

For ß2GPI no official standardization exists so far. The results are expressed in arbitrary EliA Units/ml with a cut-off at 10 EliA U/ml.

However, there are monoclonal antibodies available which are in discussion to be used as international standards ("Sapporo-Monoclonals"– Ichikawa K et al, Arthritis Rheum 1999;42:2461-70). Both Sapporo Monoclonals (IgG, IgM) work in the EliA system. An amount of 3.48 ng/ml of the Sapporo Monoclonal HCal gives a signal in EliA β2-Glycoprotein I IgG, which equals 10 EliA U/ml. An amount of 17.36 ng/ml of the Sapporo Monoclonal EY2C9 gives a signal in EliA β2-Glycoprotein I IgM, which equals 10 EliA U/ml.

Measured with ELiA Cardiolipin IgG, the Sapporo Monoclonal HCal also gives a very good signal, while in ELiA Cardiolipin IgM the Sapporo Monoclonal EY2C9 is not detected.

EliA™ Cardiolipin in NEQAS samples



Samples from the international NEQAS quality assessment scheme which have been collected over the last six years were checked in EliA Cardiolipin IgG and results were compared to the designated targets. Figure 7 shows the mean results of each target group. Table 2 shows the results using simplified categories. EliA Cardiolipin perfectly meets the requirements and expectations.

Figure 7: *Mean results of sera from each target group.*

Perfect correlation with results from quality assessment schemes

	EliA Cardiolipin IgG			
NEQAS Target	**negative (< 10)**	**weak positive (10 – 40)**	**strong positive (> 40)**	**Total**
negative	18	0	0	18
weak positive	0	6	0	6
mod. positive	0	2	6	8
strong positive	0	0	5	5

Table 2: *Performance of EliA Cardiolipin IgG in retrospectively checked samples from a quality assessment scheme. The NEQAS target categories were combined for easier interpretation: ´weak / moderate positive´ was included in ´weak positive´and ´moderate / strong positive´ (see figure 7) was included in ´strong positive´.*

Excellent Reproducibility

Variability is known to be a critical aspect in APS testing. Due to the fully automated, standardized workflow, the EliA Cardiolipin and EliA β2-Glycoprotein I tests show a highly consistent and robust performance. Detailed analysis of coefficients of variation in various runs on several Phadia 250 instruments are shown in tables 3 – 6. The statistical evaluation was performed by Analysis of Variance according to the NCCLS guidelines.

Robustness and high precision for consistent results

Sample	Mean Value	CV (%)	
		Intra-Run	Inter-Run
1	8.0 GPL	3.9	2.8
2	47.2 GPL	3.1	2.4
3	80.3 GPL	3.8	2.8

Sample	Mean Value	CV (%)	
		Intra-Run	Inter-Run
1	24.0 MPL	3.3	5.4
2	53.0 MPL	3.7	3.2
3	105.1 MPL	3.5	3.2

Table 3– 4: *Precision of EliA Cardiolipin IgG (Table 3) and EliA Cardiolipin IgM (Table 4).*

Sample	Mean Value	CV (%)	
		Intra-Run	Inter-Run
1	15.5 U/ml	3.8	4.7
2	43.0 U/ml	3.0	3.7
3	112.1 U/ml	3.1	4.5

Sample	Mean Value	CV (%)	
		Intra-Run	Inter-Run
1	18.2 U/ml	1.9	2.7
2	58.6 U/ml	3.0	4.3
3	196.5 U/ml	3.9	4.0

Table 5– 6: *Precision of EliA β2-Glycoprotein I IgG (Table 5) and EliA β2-Glycoprotein I IgM (Table 6).*

Technical Data

Products	EliA™ Cardiolipin IgG, EliA™ Cardiolipin IgM EliA™ β2-Glycoprotein I IgG, EliA™ β2-Glycoprotein I IgM
Antigens	
EliA™ Cardiolipin	bovine cardiolipin and bovine β2-glycoprotein I
EliA™ β2-Glycoprotein I	human purified β2-glycoprotein I
Standardisation / Calibration	6 point IgG or IgM standard curves EliA™ Cardiolipin is calibrated against a set of established standard sera ("Harris sera"); results in GPL-U/ml and MPL-U/ml, respectively

Cut-off / measuring range	negative	weak positive	positive	measuring range
EliA™ Cardiolipin IgG (in GPL-U/ml)	< 10	10 - 40	> 40	0.5 – ≥ 418
EliA™ Cardiolipin IgM (in MPL-U/ml)	< 10	10 - 40	> 40	0.8 – ≥ 472
	negative	equivocal	positive	measuring range
EliA™ β2-Glycoprotein I IgG (in EliA U/ml)	< 7	7 – 10	> 10	0.6 – ≥ 532
EliA™ β2-Glycoprotein I IgM (in EliA U/ml)	< 7	7 – 10	> 10	0.9 – ≥ 576

Dilution	1:10 (automated) for EliA™ Cardiolipin IgG, EliA™ Cardiolipin IgM, and EliA™ β2-Glycoprotein I IgG, 1: 50 (automated) for EliA™ β2-Glycoprotein I IgM
Sample Material	Serum, Plasma (EDTA, citrate, heparin)

Ordering Information

	Package size	*Article No.*
EliA™ Cardiolipin™ IgG Well	4 x 12	14-5529-01
EliA™ Cardiolipin™ IgM Well	4 x 12	14-5530-01
EliA™ β2-Glycoprotein I IgG Well	4 x 12	14-5532-01
EliA™ β2-Glycoprotein I IgM Well	4 x 12	14-5533-01
EliA™ Controls		
EliA™ APS Positive Control 100	6 vials	83-1054-01
EliA™ APS Positive Control 250	6 vials	83-1055-01
EliA™ IgG/IgM/IgA Negative Control 100	6 vials	83-1042-01
EliA™ IgG/IgM/IgA Negative Control 250	6 vials	83-1037-01

For EliA™ specific reagents and general reagents please refer to the Phadia product catalog.

Phadia

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