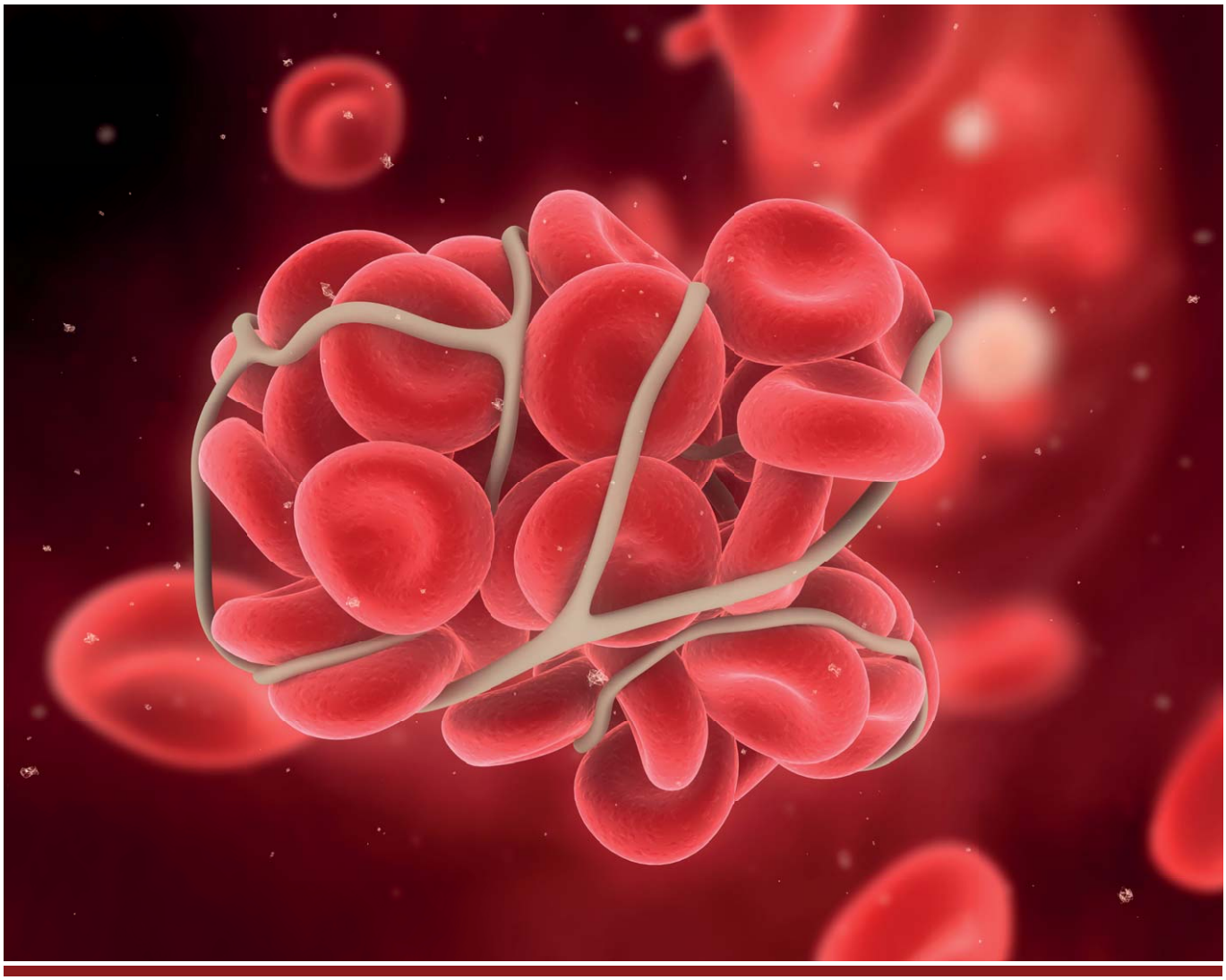
EUROIMMUN Medizinische Labordiagnostika AG

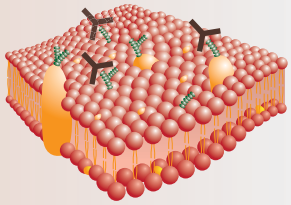
Anti-Phospholipid Syndrome

EUROIMMUN ELISAs for the determination of autoantibodies against phospholipids



- **Highly specific – also in difficult control panels (e.g. lues, hepatitis and parvovirus B19)**
- **High sensitivity in accordance with antibody prevalences from international meta studies (Cevera et al., 2002)**
- **Individual availability of all Ig classes recommended by the international consensus statement (Lakos et al., 2012)**
- **Reliable and full automation using the EUROIMMUN Analyzer I or I-2P**

Phospholipids



"Phospholipids" is an umbrella term for various kinds of phosphoric lipids. They constitute the **main part of the cell membrane** in the form of a double lipid layer. Furthermore, they act as reactive surfaces in **blood coagulation**, thus allowing the formation of multi-enzyme complexes in the coagulation cascade. The most relevant phospholipids include cardiolipin and phosphatidylserine.

Anti-phospholipid antibodies

Anti-phospholipid antibodies (APLA) are a very heterogeneous group of antibodies, which are directed against different phospholipids or plasma proteins. Among the principal target antigens are cardiolipin and phosphatidylserine, but also proteins such as β_2-glycoprotein 1 or prothrombin, which act as phospholipid-binding cofactors. In some cases the antibodies are directed against neoepitopes, which are produced during the formation of a complex, for instance of cardiolipin and β_2-glycoprotein 1. In suspected anti-phospholipid syndrome there are mainly three diagnostically relevant substance classes: **antibodies against cardiolipin and β_2-glycoprotein 1 (β_2GP1**) , and lupus anticoagulant.

Anti-phospholipid syndrome

Anti-phospholipid syndrome (APS) is an autoimmune disease. The body produces antibodies against phospholipids and associated proteins, which can cause a wide range of clinical symptoms. Characteristic symptoms include **increased thrombotic tendency** and **pregnancy complications**. Vascular occlusions can occur both in veins and arteries. Leg vein thrombosis and lung embolism are the most frequent. APS in pregnant women is associated with a significantly higher risk of compli-cations such as spontaneous abortion or premature delivery. In cases of repeated miscarriages without any noticeable cause APS should be taken into consideration.

The syndrome is divided into primary and secondary APS. Primary APS is not accompanied by any other disease. If APLA are found in combination with other autoimmune diseases, the term "secondary APS" applies. This form is mainly found in collagenoses – most often in systemic lupus erythematosus, and more rarely in scleroderma or Sjögren's syndrome.

Test systems

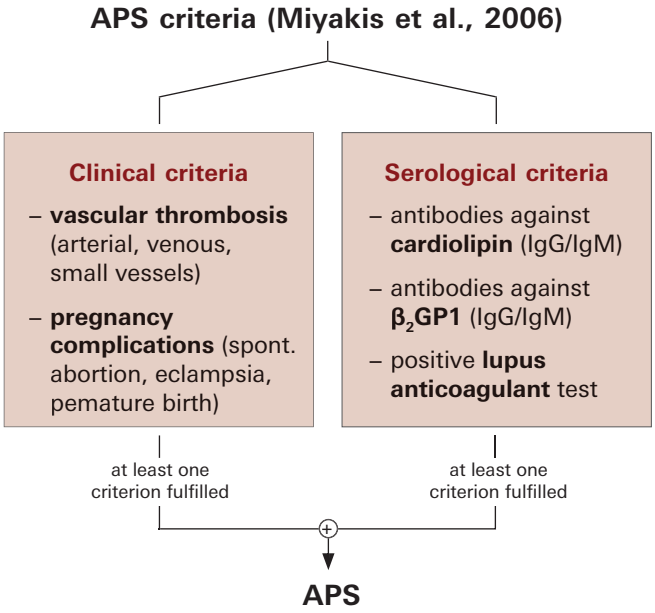
ELISA is the method of choice for the detection of APLA, since it is highly sensitive, simple to perform and does not require fresh plasma. EUROIMMUN offers **microtiter ELISAs** for quantitative determination of autoantibodies against **cardiolipin, β_2-glycoprotein 1 and phosphatidylserine**. The immunoglobulin classes IgA, IgG and IgM can be investigated separately or together (IgAGM).

Alternatively, lupus anticoagulant can also be determined by measuring the extension of the coagulation time. These test systems have a high specificity for APS, but they are less sensitive than autoantibody ELISAs. Moreover, the test is complex and laborious in contrast to APLA ELISAs, which can be performed both manually and by fully automated systems.

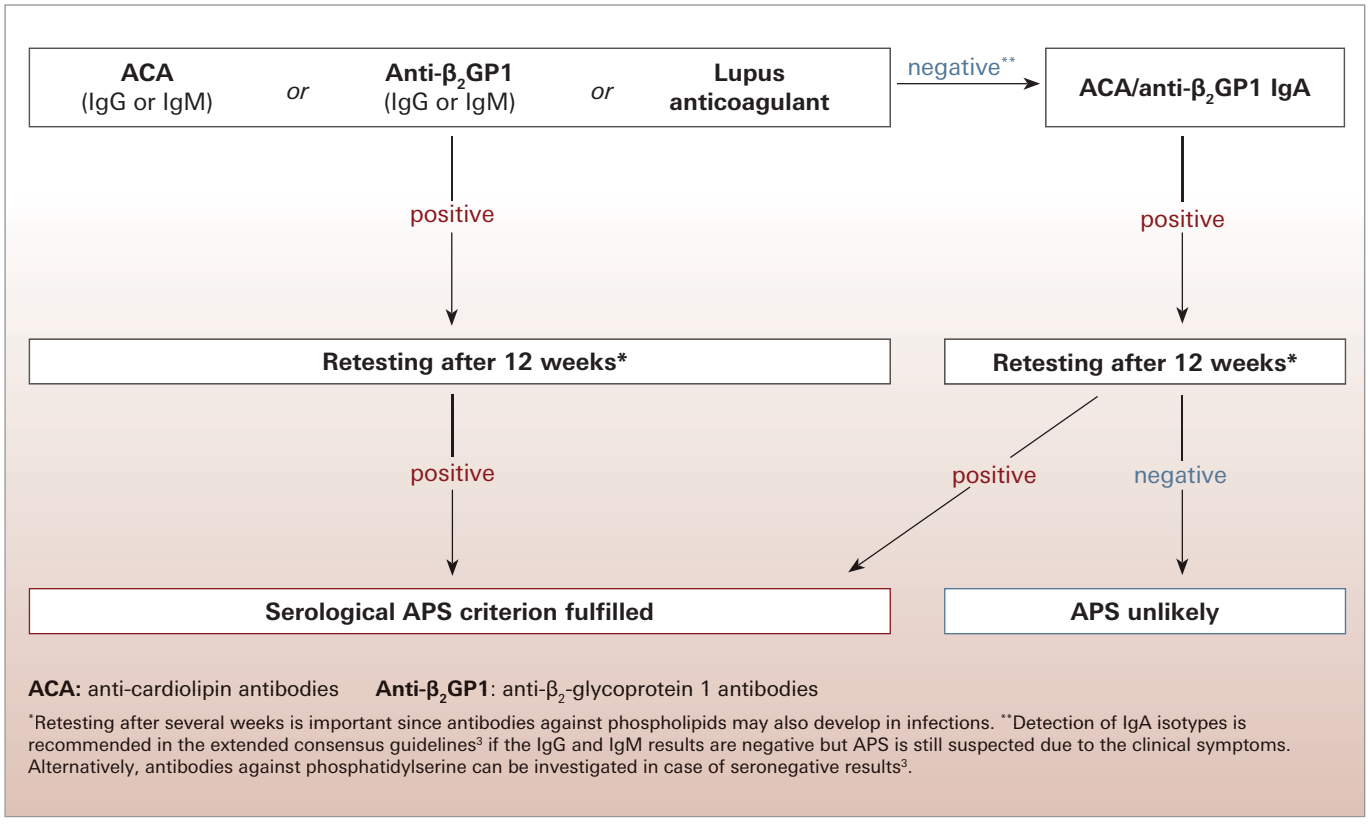
APS classification criteria

The first official classification criteria for anti-phospholipid syndrome were drafted in 1998 at a workshop at the 8th International Symposium on Anti-Phospholipid Antibodies in Sapporo, Japan (**"Sapporo criteria"**)[1]. According to these criteria, APS can be considered proven if at least one clinical and one serological criterion are met. When updating the criteria in 2006 (**"Miyakis criteria"**)[2] antibodies against β_2-glycoprotein 1 were added (see figure on the right).

For the **fulfilment of serological criteria** the detection of at least one of the following parameters is recommended: **antibodies against cardiolipin** or **β_2-glycoprotein 1 (Ig classes G or M)** or a positive **lupus anticoagulant (LA) result**. According to the classification criteria the serological result should be confirmed by a second test after 12 weeks. In the extended criteria3 from 2012 it is further recommended that antibodies of class IgA be also investigated if a negative result is yielded for ACA or anti-β_2GP1 (see figure below).



Diagnostic strategy



ACA: anti-cardiolipin antibodies **Anti-β_2GP1**: anti-β_2-glycoprotein 1 antibodies

*Retesting after several weeks is important since antibodies against phospholipids may also develop in infections. **Detection of IgA isotypes is recommended in the extended consensus guidelines[3] if the IgG and IgM results are negative but APS is still suspected due to the clinical symptoms. Alternatively, antibodies against phosphatidylserine can be investigated in case of seronegative results[3].

References:

[1] Wilson W et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome, Arthritis Rheum 1999

[2] Miyakis S et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J Thromb Haemost 2006

[3] Lakos G et al. International Consensus Guidlines on Anticardiolipin and Anti-β2-Glycoprotein I Testing, Arthritis Rheum 2012

Study data on EUROIMMUN test systems

EUROIMMUN ELISAs for the detection of antibodies against cardiolipin and β_2GP1 show a very high specificity in clinical studies. Only 0 to 2% of sera from patients with viral hepatitis or parvovirus B19 infections and from healthy blood donors were positive. In studies on competitive test systems these values were significantly higher (up to 50%).

APLA can occur in connection with syphilis, which explains the somewhat high occurrence of ACA and anti-β_2GP1 antibodies. The presence of both autoantibodies in APS and SLE corresponds to the data found in recent literature. The agreement was particularly high for prevalences of ACA in an international meta study[4].

[4]Cervera R. et al., Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients; Arthritis and Rheumatism 2002

Panel	n	ACA positive (IgG and/or IgM)	Anti-β_2GP1 positive (IgG and/or IgM)	Literature reference
APS	26	86%	86%	40-90%
SLE	347	24%	25%	20-40%
Syphilis	45	11%	13%	up to 50%
Viral hepatitis	336	2%	1%	up to 50%
Anti-parvovirus B19 positive	42	0%	not determined	20-30%
Healthy blood donors	504	0.6%	0.4%	up to 12%

Comparison of the EUROIMMUN Anti-Cardiolipin ELISA with competitive test systems

A comparison study on the anti-cardiolipin ELISAs from two competitors revealed that the EUROIMMUN ELISA has the highest specificity (100%) at the same sensitivity (93%).

*borderline sera were classified as positive, **borderline sera were classified as negative

Anti-Cardiolipin ELISA (IgG and/or IgM)		
	Specificity	**Sensitivity**
EUROIMMUN	100%	93%
Competitor A	96%	93%
Competitor B	82*/59**%	93*/86**%

Specificity of the Anti-Cardiolipin ELISA (IgG and/or IgM)			
Controls (n = 142)		**EUROIMMUN**	
		positive	**negative**
Competitor A	**positive**	**0**	**6**
	negative	**0**	**136**
Competitor B	**positive**	**0**	**25**
	borderline	**0**	**34**
	negative	**0**	**83**

Sensitivity of the Anti-Cardiolipin ELISA (IgG and/or IgM)			
APS (n = 29)		**EUROIMMUN**	
		positive	**negative**
Competitor A	positive	26	1
	negative	1	1
Competitor B	positive	25	0
	borderline	1	1
	negative	1	1

Prevalences of autoantibodies against cardiolipin and β_2GP1 in patients with APS

In a study, 86% of APS patients could be identified by determining ACA and anti-β_2GP1 antibodies of classes IgA, IgG and IgM. The additional determination of IgA yielded an increase in the serological detection rate for anti-β_2GP1 by 19% compared to the determination of IgM and IgG. The highest sensitivity could be achieved by simultaneous investigation of ACA and anti-β_2GP1 antibodies, allowing the serological detection of 100% of APS patients (data not presented).

Ig class	Prevalence	
	ACA	**Anti-β_2GP1**
IgA:	0%	19%
IgG	48%	10%
IgM	10%	19%
IgG + IgM	86%	67%
IgA + IgG + IgM	86%	86%

Product overview

All three ELISA systems have the same incubation conditions and times. They can be combined on a microplate for the determination of different immunoglobulin classes.

Antibodies against	Ig class	Order number
Anti-cardiolipin	IgA, IgG, IgM, IgAGM	EA 1621-9601 A, G, M or P
Anti-β_2-glycoprotein 1	IgA, IgG, IgM, IgAGM	EA 1632-9601 A, G, M or P
Anti-phosphatidylserine	IgA, IgG, IgM, IgAGM	EA 162a-9601 A, G, M or P