



Antiphospholipid antibody testing at Melbourne Pathology

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- The antiphospholipid (antibody) syndrome (APS) is characterised by vascular thromboses and fetal loss underpinned by procoagulant antiphospholipid autoantibodies.
- The term 'antiphospholipid' antibodies is actually a misnomer as the antibodies are directed to plasma proteins (such as β 2GPI) that bind phospholipids *in vitro*.
- In suspected APS, lupus anticoagulant, cardiolipin antibodies (IgG) and β 2GPI antibodies (IgG) should be requested. If 'antiphospholipid antibodies' are requested, Melbourne Pathology will perform all three.

The clinical spectrum of vascular thromboses may involve venous and/or arterial beds while the obstetric complications range from recurrent fetal loss to pre-eclampsia.

The international APS classification criteria recognise these clinical manifestations supported by laboratory criteria. In suspected cases of unexplained vascular thrombotic events and obstetric adverse events, tests for antiphospholipid antibodies (aPL) may be useful for diagnosis and to guide therapeutic decisions regarding anticoagulation.

The term 'antiphospholipid' antibodies is actually a misnomer as the antibodies are directed to plasma proteins (such as β 2GPI) that bind phospholipids *in vitro*. The antiphospholipid antibodies that can be detected in the laboratory induce the procoagulant *state in vivo*.

Tests available

Testing for aPL can be divided into:

1. solid phase assays that directly detect antibodies to cardiolipin (aCL) and antibodies to β 2Glycoprotein I (β 2GPI), and
2. liquid phase coagulation assays that indirectly detect functional antibodies, referred to as the Lupus Anticoagulant (LA) or lupus inhibitor. This is also a misnomer, as the presence of LA has a low specificity for the presence of lupus erythematosus. Furthermore, despite causing prolongation of coagulation assays *in vitro*, LA is **procoagulant** *in vivo*.

Anticoagulation may affect the LA assay, particularly newer oral agents (Pradaxa, Rivaroxiban, Apixaban, Edoxaban), Clexane and unfractionated heparin, but does not affect solid phase aPL assays.

aCL and β 2GPI antibodies of the IgG isotype have the strongest association with clinical manifestations of the syndrome. IgM antibodies (aCL and β 2GPI) and antibodies of other isotypes have a much weaker association and some studies even suggest a negative association with disease manifestations. For that reason, testing for IgM β 2GPI and IgM aCL are no longer recommended.¹

Isolated positive results against aPL antibodies other than aCL or β 2GPI IgG have been rarely demonstrated but have uncertain clinical utility. As such, these tests remain in the research domain and are not included in routine APS screening tests.

Classification criteria (see table overleaf)

Classification criteria for APS were developed for patient inclusion in clinical studies. The criteria have imperfect performance as diagnostic criteria. One of the difficulties is that aPL testing is poorly standardised across laboratories and the result of this testing is critical to the classification of patients. Furthermore, it is not possible to calculate meaningful sensitivity and specificity estimates of aPL as the presence of the antibodies is part of the classification criteria which are the gold standard.

The presence of aPL may be more usefully considered as risk factors for the clinical features of APS. In support of this concept, data are emerging that show patients with more than one positive aPL have higher risk of thrombotic complications. ie. Patients who are LA, aCL and β 2GPI antibody positive have the highest risk.²

Importantly, aPL antibodies may be identified in asymptomatic patients without recognised clinical manifestations.

Therefore, as with all autoantibody testing, testing for antiphospholipid antibodies should be undertaken when APS is clinically suspected.





APS is present if at least one of the following clinical criteria and one of the laboratory criteria are met

1. Clinical criteria

- a. Vascular thrombosis confirmed by objective validated criteria
- b. Pregnancy morbidity any one of:
 - i. unexplained death of morphologically normal fetus beyond 10th week gestation
 - ii. premature birth of morphologically normal neonate before 34th week gestation due to eclampsia, severe pre-eclampsia or placental insufficiency
 - iii. three or more unexplained consecutive spontaneous abortions before 10th week gestation, excluding anatomic, hormonal and chromosomal causes

Laboratory criteria (must be detected on two or more occasions at least 12 weeks apart)

Any one of:

- i. LA detected according to international guidelines
- ii. aCL antibody, present in medium or high titre
- iii. a β 2GP1 antibody

Table 1. Summary of classification criteria for APS (Miyakis et al.)

These criteria are included as a guide only. As above, in clinical practice, some patients will have APS without fulfilling these criteria.

It is important to demonstrate persistent autoantibodies over time, as transient aPL antibodies are common and of uncertain clinical significance. It is common practice to demonstrate persistent aPL antibodies as APS treatment usually requires long term anticoagulation.

Usual testing panel

In suspected APS, lupus anticoagulant, cardiolipin antibodies (IgG) and β 2GPI antibodies (IgG) should be requested. If 'antiphospholipid antibodies' are requested, Melbourne Pathology will perform all three. As β 2GPI antibodies are a subset of aCL antibodies, and are included in APS classification criteria, requests for aCL antibodies will have aCL and β 2GPI antibodies performed. These tests are covered by Medicare.

Interpretation

IgG aCL antibodies >40 GPL-U/ml and IgG β 2GPI >10 U/ml are considered positive (medium or high titre as per the above criteria). Higher concentration antibodies correlate with a higher risk of APS clinical features.

Furthermore, as above, the finding of more than one aPL specificity is associated with higher risk of future APS clinical manifestations.

References

1. Favoloro Pathology 46(6). October 2014
2. Galli et al. 2012
3. Figure 1: "Thrombotic microangiopathy: new insights" Curr Opin Nephrol Hypertens 19 (3): 242-7 (May 2010).



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After graduating from Monash University in 1997, Dr Unglik trained at the Royal Melbourne and Alfred Hospitals. He obtained combined fellowship with both the Royal Australasian College of Physicians and the Royal College of Pathologists of Australasia in 2007.

After completing advanced training he was appointed to the Department of Clinical Immunology and Allergy at the Royal Melbourne Hospital where he was also Head of the Immunopathology laboratory unit until 2015.

Dr Unglik joined Melbourne Pathology in February 2010 as a Consultant Immunopathologist. He continues as a Consultant Clinical Immunologist and Allergist in the Department of Clinical Immunology and Allergy at the Royal Melbourne Hospital.

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Dr Bosco completed his Bachelor of Medicine and Bachelor of Surgery with Honours in 2002 at the University of New South Wales. From 2007 – 2011 he trained in Clinical Immunology/Allergy and Immunopathology in New South Wales and Victoria, at the Westmead and Royal Melbourne Hospitals.

Since being awarded his Fellowship with the Royal Australasian College of Physicians and Royal College of Pathologists of Australasia in 2011, Dr Bosco has held Clinical Immunology and Allergy consultant positions at both the Royal Victorian Eye and Ear and the Alfred hospitals. He also works as a consultant Immunologist and Allergist with the Epworth Allergy Specialists and as an Immunopathologist with Alfred Pathology. He completed his PhD on the immunoregulatory properties of CD52 at the Walter Eliza Hall Institute of Medical Research.

Dr Bosco joined Melbourne Pathology as a Consultant Immunopathologist in February 2016 and has a special interest in autoantibody-associated autoimmune disease, allergic disease and immunodeficiency.