Antiphospholipid Syndrome

Tools to Aid in the Accurate Diagnosis of Antiphospholipid Syndrome

Inova Diagnostics
A Werfen Company

Experts in autoimmunity

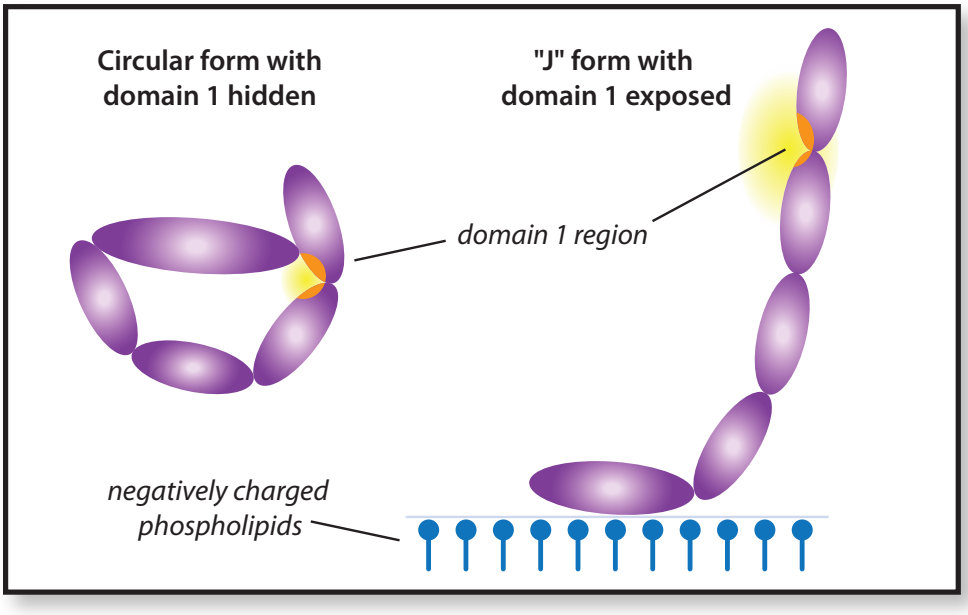
Inova Diagnostics provides a comprehensive menu of products to aid in the diagnosis of Antiphospholipid Syndrome.

QUANTA Lite® ELISA tests are high quality reagents which are compatible with a variety of automation solutions offered through Inova Diagnostics. **QUANTA Flash®** tests are chemiluminescent based reagents utilized on the BIO-FLASH® system. QUANTA Flash reagents offer a number of advantages including broader analytical measurement range, higher sensitivity and precise quantitation, giving clinicians added clarity in the diagnosis of APS.

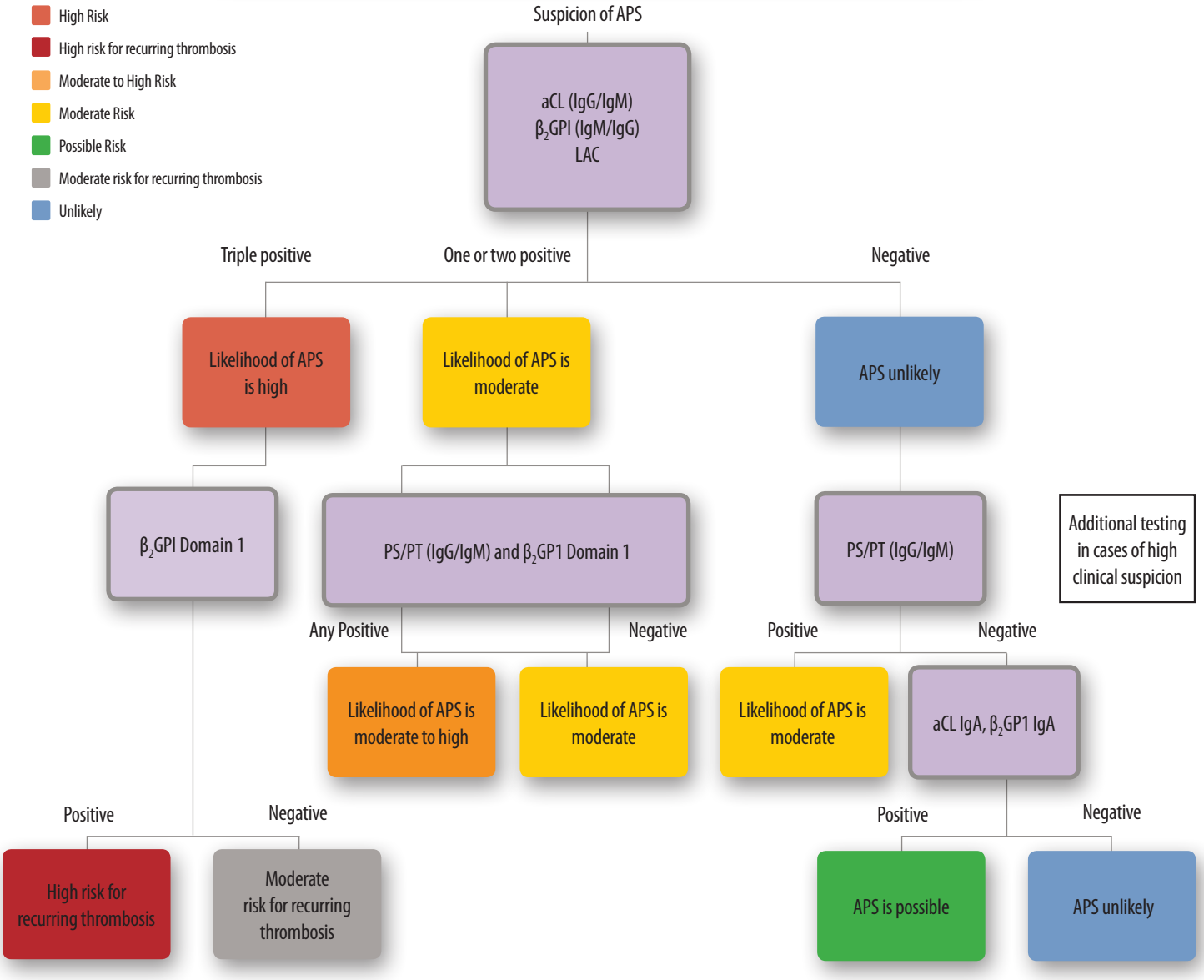
Inova Diagnostics offers the most advanced APS tests including QUANTA Lite PS/PT and QUANTA Flash β_2GP1 Domain 1. In cases where LAC (lupus anticoagulant) testing results are ambiguous but the suspicion of APS is high, the presence of anti-PS/PT antibodies may greatly aid in clarifying the diagnosis due to high correlation of these antibodies to LAC. When PT (prothrombin) is bound to PS (phosphatidylserine), it's orientation changes and reveals clinically relevant epitopes distinct from assays that test for PS or PT in isolation. Antibodies to PS and PT lack specificity for APS disease manifestations while antibodies to the PS/PT complex correlate very well with both thrombotic and pregnancy complications in APS.[1-6]

The QUANTA Flash β_2GP1 Domain 1 assay, unique to Inova Diagnostics, identifies antibodies to domain 1 of β_2GP1, the immunodominant epitope in APS. The presence of anti-β_2GP1 domain 1 antibodies is associated with an increased risk for adverse events in APS including thrombosis.[7-8]

The domain 1 region of β_2 glycoprotein1 ($ß_2$GP1)



Potential Testing Algorithm for APS



Likelihood of APS based on testing outcome	Risk outcome
High	• Triple positivity is the strongest risk factor for clinical manifestations[2-6, 9]
Moderate to high	• Titers and isotype need to be considered[4-6] • Inclusion of PS/PT results in excellent diagnostic accuracy and a predictor of adverse events[2-6, 9]
Moderate	• PS/PT may identify patients with APS who are negative for serological criteria markers[10] • Positivity for at least one of the criteria markers increases likelihood for APS • Titers and isotype need to be considered (Antibodies of IgG isotype represent higher risk than that of IgM, and higher antibody level implies higher risk)[4-6]
Possible	• All criteria markers negative but one or more esoteric marker positive; APS possible, but likelihood only marginally increased[11-12] • Isolated IgA antibody positivity has been described in association with APS[9, 13-14]
Unlikely	• All markers are negative which makes APS unlikely, but can not be excluded[11-12]

Important assays you should know about

Presence of anti-PS/PT IgG or IgM antibodies indicates a higher risk category of patients

Antiphospholipid antibodies are not directed against phospholipids, but rather to phospholipid-protein complexes.

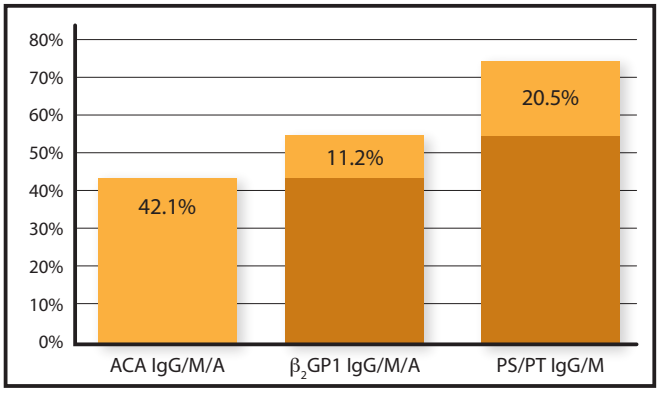
Antibodies to PS/PT correlate highly to LAC. In cases where LAC testing results are ambiguous but the suspicion of APS is high, the presence of these antibodies may greatly aid in clarifying the diagnosis. Recent evidence suggests that antibodies to the complex of PS and PT (PS/PT) identifies a distinct subset of patients with very high likelihood of adverse events.[2] Antibodies targeted to either PS or PT yield less clinically relevant information as antibodies towards the PS/PT complex.[2]

Recent studies have shown that the risk of thrombotic events increases with the number of positive test results in APS patients and aPL carriers.[2] Triple positivity for LAC, anti-β_2GP1, and PS/PT demonstrated the highest diagnostic accuracy out of 23 possible combinations of aPL tests.[2]

Anti-β_2GP1 domain 1 antibodies are highly specific for the diagnosis of APS and may help support therapeutic decision making

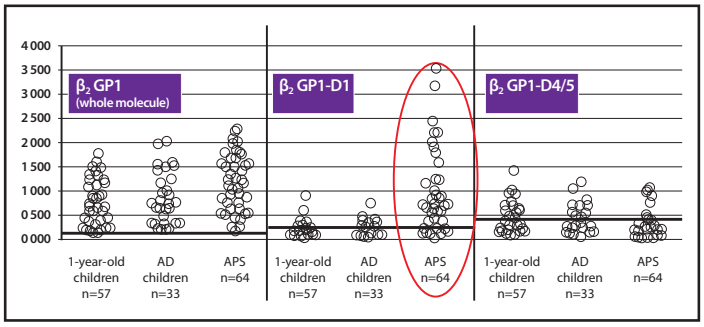
Anti-β_2GP1 antibodies target multiple epitopes in the same molecule. A growing body of evidence indicates that domain 1 is the most relevant epitope targeted by anti-β_2GP1 antibodies in patients with APS. Many studies have shown that anti-domain 1 antibodies are associated with aPL related events, both vascular and obstetric in nature.[7]

Incremental detection rates for aPL serological markers in primary APS patients (N=107)1



Sensitivity increase as assays are added

The domain 1 region of β_2GP1 does a better job differentiating APS from other diseases compared to the whole molecule or domain 4/5.



Adapted from Andreoli, et al. Ann Rheum Dis (2010)

1-year-old children: one year-old children born to mothers with systemic autoimmune diseases; AD children: children with atopic dermatitis; APS: patients with antiphospholipid syndrome.

Characteristics of criteria and non-criteria tests

According to the international consensus guidelines, a patient can be classified as having definite antiphospholipid syndrome, if one of the clinical criteria and one of the laboratory criteria listed below are present:

Clinical Criteria	Laboratory Criteria
1. Vascular thrombosis: ≥1 arterial, venous, or small vessel thrombosis 2. Pregnancy morbidity a. ≥1 fetal death (at or beyond the 10th week of gestation) b. ≥1 premature birth before the 34th week of gestation because of eclampsia, severe preeclampsia, or placental insufficiency c. ≥3 consecutive (pre) embryonic losses (before the 10th week of gestation)	1. Anticardiolipin antibody (IgG and/or IgM) in medium or high titer (i.e., >40 MPL/GPL, or above th 99th percentile), on two or more occasions at least 12 weeks apart 2. Anti-β_2-glycoprotein-I antibody (IgG and/or IgM) in medium or high titer (i.e., above the 99th percentile) on two or more occasions at least 12 weeks apart 3. Lupus anticoagulant (LAC) positivity on two or more occasions at least 12 weeks apart

Chart adapted from Miyakis M. International consensus statement on an update of the classification criteria for definite APS. Intl Soc Thromb and Haemos. 2006. 4: 295-306.

Criteria tests	
aCL IgG and IgM	• The aCL test is positive in more than 80-90% of patients with APS. However, these tests combined (IgG & IgM) offer modest specificity as this test may be positive in a variety of other disorders, including connective tissue diseases and infectious diseases such as syphilis.[15] • Association of a positive aCL test with clinical manifestations of APS occurs principally with persistent medium to high levels of aCL antibodies. IgG is more prevalent than IgM and IgA.
β_2GP1 IgG and IgM	• The β_2GP1 test is more specific than aCL and should be used in conjunction with aCL tests. • Omission of the β_2GP1 test can result in failure to correctly classify as much as 30% of APS patients.[17]
LAC	• The LAC test measures the ability of antiphospholipid antibodies to prolong phospholipid-dependent clotting reactions. • The antiphospholipid antibodies detected in aCL and LAC are specific for phospholipids, phospholipid-binding proteins, or a complex of these molecules. These antibodies are heterogeneous; the two sets (aCL and LAC) do not necessarily identify the same antibodies. • This test is not recommended for use when patients are under anti-coagulation therapy as these drugs may interfere with LAC test results. • Due to complexity and varied protocols for LAC, this test may be subject to variability of results.[18]
Non-criteria tests	
aCL IgA	• Genetic and ethnic background can impact the role of testing for IgA aCL, for example African-Americans, Afro-Caribbean, and Hispanic patients with SLE may have elevated levels of IgA aCL.[16] • IgA aCL was the only aCL isotype present in 82% of aCL-positive Afro-Caribbean patients.[16]
β_2GP1 IgA	• Testing for IgA β_2GP1 is recommended when other aPL assays are negative and APS is suspected. • Positivity for IgA β_2GP1 is more common in women who experience unexplained recurrent spontaneous abortion and unexplained fetal death even when test results for other isotypes and LAC are negative.[16]
PS/PT IgG and IgM	• A systematic review of data from over 7000 patients shows that: - When PT binds with PS, a conformational change occurs in PT allowing clinically relevant epitopes to be expressed; antibodies directed towards these epitopes are distinct from either PS or PT antibodies alone.[20] - Routine measurement of PS/PT, but not PT or PS, is useful in establishing the thrombotic risk of patients.[20]
β_2GP1 Domain 1	• Domain 1 of β_2GP1, the immunodominant epitope in APS, becomes exposed when bound to negatively charged phospholipids. • Test results for Domain 1 of β_2GP1 may help guide clinical decision making given the marker's strong association with increased risk of thrombosis. • High titer anti-β_2GP1 domain 1 antibodies are associated with a greater risk of thrombosis.[7]

Comprehensive offering of assays

Method	Name
QUANTA Lite ELISA	aCL IgG
	aCL IgM
	aCL IgA
	aCL Screen
	β_2GP1 IgG
	β_2GP1 IgM
	β_2GP1 IgA
	β_2GP1 Screen
	PS/PT IgG
	PS/PT IgM
	Phosphatidylserine IgG
	Phosphatidylserine IgM
	Phosphatidylserine IgA

Method	Name
QUANTA Flash CIA	aCL IgG
	aCL IgM
	aCL IgA
	β_2GP1 IgG
	β_2GP1 IgM
	β_2GP1 IgA
	β_2GP1 Domain 1

For more information contact your Inova representative or call customer service at +1-858-586-9900, or visit our website at www.inovadx.com

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www.inovadx.com

San Diego, CA 92131 USA
PH: +1-858-586-9900 US Toll Free: 1-800-545-9495 FAX: +1-858-586-9911

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