Advancements in Antiphospholipid Syndrome (APS) Testing:

Antibodies to Beta-2 Glycoprotein1-Domain1 (β2GP1-D1) and Anti-phosphatidylserine-prothrombin (aPSPT)

by David Corwin, M.D. and Joel Adams, MLS (ASCP)SM, Kevin Geibel, MT (ASCP), Ryan Cronrath, MLS (ASCP)SM

CLINICAL BACKGROUND

Antiphospholipid Syndrome (APS) is a systemic autoimmune disorder, characterized by recurrent arterial or venous thrombosis, and/or early and late stage miscarriages. Anticardiolipin antibodies (aCL) detected by ELISA and lupus anticoagulant (LAC) detected by clotting assays have been the most established and standardized tests for the diagnosis of APS. Antiphospholipid antibodies (aPL) also represent a large group of immunoglobulins of considerable clinical importance due to their association with APS, neurological disorders, pulmonary hypertension and thrombocytopenia.7 The association between anti-prothrombin (aPT) and LAC has been studied widely. Recently it has been hypothesized that prothrombin binds to anionic phospholipids exposed on the endothelial cells and that aPT activates endothelial cells to induce procoagulant substances via prothrombin.2 We now know that the antibodies most closely associated with APS and the LAC are directed towards a complex of anionic phospholipids such as phosphatidylserine-prothrombin (PSPT) rather than PS or PT alone.1,4,5

In addition to aPSPT, antibodies directed against Domain 1 of the Beta-2 Glycoprotein 1 molecule (β2GP1-D1) have been shown to be useful in assessing the risk of thrombosis. Beta-2 Glycoprotein 1, also called apolipoprotein H, is a 44 kDa glycoprotein which has five domains and is present in plasma. The fifth domain contains a cluster of positively charged amino acids which is responsible for the binding to anionic phospholipids. APS patients can produce antibodies against several epitopes of the molecule and recently, an association between antibodies to epitope on domain 1 (D1) and vascular events has been described.3 It’s important to note, however, that β2GP1-D1 is not intended to replace assays for antibodies against the whole β2GP1 molecule which is still required according to the classification criteria for APS.8

CLINICAL APPLICATION

In light of the clinical significance of aPSPT and β2GP1-D1, and in order to adhere to the newest recommendations from the International Society on Thrombosis and Haemostasis (ISTH), we offer aPSPT IgG and IgM, and β2GP1-D1 individually, as well as two panels: "Antiphospholipid Syndrome Panel, Basic Reflex" [APSBAS] and "Antiphospholipid Syndrome Panel, Comprehensive Reflex" [APSCOM].

The “Antiphospholipid Syndrome Panel, Basic RFLX” [APSBAS] mirrors current ISTH APS diagnostic guidelines, and includes the APS criteria tests: LAC, aCL IgG and IgM, and B2GP1 IgG and IgM. The Basic Panel should be the first-line testing ordered by clinicians who suspect APS. If one or more of the assays in this panel are positive, then the recommended follow-up testing is aPSPT IgG and IgM. If the LAC, at least 1 of the aCL antibodies, and at least 1 of the β2GP1 antibodies are positive, then β2GP1-D1 is also recommended in addition to aPSPT testing. If the patient does test positive for LAC, either aCL antibody, and either β2GP1 antibody, the patient is considered to have met the laboratory criteria for APS diagnosis, but these laboratory findings taken by themselves only assess the probability of diagnosis.

Continued on P.2
The "Antiphospholipid Syndrome Comprehensive Panel" [APSCOM], contains the same criteria tests as the basic panel, but also includes the second-line testing, aPSPT IgG and IgM, and B2GP1-D1 all in one panel. The Comprehensive Panel is useful to help confirm APS in patients who are suspected of having the syndrome (known autoimmune disease, history of thrombosis and/or miscarriage), but may not test positive with first line tests. In accordance with the ISTH recommendations, overall risk of APS and thrombotic event must always be evaluated based on the clinical picture and other laboratory findings (see below). If overall results do not fit the clinical picture, repeat testing may be indicated, as antibody levels may fluctuate with time. Repeat evaluation is suggested in 12 or more weeks to confirm positive results, ideally in the absence of anticoagulant therapy.8

Revised Classification Criteria for Antiphospholipid Antibody Syndrome8
(At least 1 clinical and 1 laboratory criterion must be met)

**CLINICAL CRITERIA**
- Vascular thrombosis – one or more clinical episodes of arterial, venous, or small-vessel thrombosis in any tissue or organ validated by imaging studies or histopathology
- Pregnancy morbidity
- One or more unexplained deaths of a morphologically normal fetus after 10th week of gestation
- One or more premature births of a morphologically normal neonate before the 34th week of gestation due to preeclampsia, eclampsia, or placental insufficiency
- Three or more unexplained, consecutive, spontaneous abortions before 10th week of gestation, and with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded

**LABORATORY CRITERIA**
- Positive test on 2 or more occasions at least 12 weeks apart
- Lupus anticoagulant – detected in plasma according to the guidelines of the International Society on Thrombosis and Hemostasis
- Anticardiolipin (aCL) – IgG and/or IgM isotype present in a medium or high titer (>99th percentile of normal population)
- Anti-Beta-2 Glycoprotein 1 (anti-β2GP1) – IgG and/or IgM isotype in a medium or high titer (>99th percentile)

**CLINICAL INDICATIONS FOR TESTING**

Vascular Thrombosis
- One or more clinical episodes of arterial, venous, or small-vessel thrombosis in any tissue or organ validated by imaging studies or histopathology.

Pregnancy Morbidity
- One or more unexplained deaths of a morphologically normal fetus after 10th week of gestation.
- One or more premature births of a morphologically normal neonate before the 34th week of gestation due to preeclampsia, eclampsia, or placental insufficiency.
- Three or more unexplained, consecutive, spontaneous abortions before 10th week of gestation, and with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

ORDER: APSBAS — APS Panel, Basic
- Lupus Anticoagulant Reflex Panel (LAC)
- Cardiolipin Antibodies IgG and IgM (aCL)
- Beta-2 Glycoprotein 1 Antibodies (B2GP1)

ORDER: PSPTGM
- Negative
- Positive for one or both

APS Unlikely
- Suggest retest in 12 weeks if APS still suspected

Possible APS
- Suggest retest in 12 weeks if APS still suspected to prove persistence
- Consider referral to specialist

APS Likely
- Suggest retest in 12 weeks if APS still suspected to prove persistence
- Consider referral to specialist

**APSCOM — APS Panel, Comprehensive**
- Confirm APS in patients suspected of having APS (known autoimmune disease, or history of clinical indications above).
- Follow-up patients who test negative with first line testing (APSBAS), but are suspected of having APS.
## ANTIPHOSPHOLIPID SYNDROME PANELS

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>APSBAS</th>
<th>APSCOM</th>
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<tbody>
<tr>
<td>Antiphospholipid Syndrome, Basic, Reflex</td>
<td>Lupus Anticoagulant (LAC)</td>
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<tr>
<td></td>
<td>Anticardiolipin IgG</td>
<td>Anticardiolipin IgG</td>
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<td></td>
<td>Anticardiolipin IgM</td>
<td>Anticardiolipin IgM</td>
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<td></td>
<td>Beta2 Glycoprotein1 IgG</td>
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<tr>
<td></td>
<td>Beta2 Glycoprotein1 IgM</td>
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**CONTAINS**
- Lupus Anticoagulant (LAC)
- Anticardiolipin IgG
- Anticardiolipin IgM
- Beta2 Glycoprotein1 IgG
- Beta2 Glycoprotein1 IgM

**METHOD**
- LAC: clotting assays
- Cardiolipins/B2GP1: Chemiluminescent immunoassay (CIA)

**CPT CODES**
- 86146 x2, 86147 x2, 85670, 85613, 85610, 85730
- 86148 x2, 86146 x3, 86147 x2, 85670, 85613, 85610, 85730

**SPECIMEN REQUIREMENTS**
- **CIA**
  - Preferred Specimen: Serum
  - Preferred Volume: 1.5 mL
  - Minimum Volume: 1.0
  - Store and Transport: Refrigerated

- **Clotting Assays**
  - Preferred Specimen: Frozen plasma from blue top tube
  - Preferred Volume: 4 mL
  - Minimum Volume: 2 mL
  - Store and Transport: Frozen

**SCHEDULE**
- Tue-Sat (PAML), Tue-Sat (PSHMC)
- Tue-Sat (PAML), Tue-Sat (PSHMC)

**TURNAROUND TIME**
- 2-4 Days

## ADDITIONAL TESTS FOR ANTIPHOSPHOLIPID SYNDROME

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<tr>
<th>DESCRIPTION</th>
<th>PSPTGM</th>
<th>B2GPD1</th>
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<tbody>
<tr>
<td>Antiphosphatidylserine-prothrombin IgG and IgM</td>
<td>Chemiluminescent immunoassay (CIA)</td>
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**ORDER CODE**
- PSPTGM
- B2GPD1

**METHOD**
- Enzyme linked immunosorbent assay (ELISA)
- Chemiluminescent immunoassay (CIA)

**CPT CODES**
- 86148 x2
- 86146

**SPECIMEN REQUIREMENTS**
- Preferred Specimen: Serum
- Preferred Volume: 0.750 mL
- Minimum Volume: 0.5 mL
- Store and Transport: Refrigerated

**SCHEDULE**
- Tue, Thur, Sat
- Tue, Thur, Sat

**TURNAROUND TIME**
- 2-4 Days
- 2-4 Days
SELECTED REFERENCES


For more information, please contact your local sales representative.