

## Anti-phospholipid Antibody Testing (Lupus Anticoagulant Testing)

### Background Information

Anti-phospholipid syndrome (APS) is the most common cause of acquired thrombophilia, and the presence of antiphospholipid antibody (APA) is associated with significant morbidity and mortality across diverse patient populations. Both primary and secondary forms of APAs exist, the difference being whether they arise spontaneously or in association with another condition. These antibodies — also known as lupus anticoagulants due to their prevalence in patients with systemic lupus erythematosus — are extremely heterogeneous and are directed against a wide variety of anionic phospholipids, including cardiolipin,  $\beta$ 2 glycoprotein 1 (B2GP1), cell-membrane phosphatidylserine, and many others. Paradoxically, APAs prolong clot-based assays in vitro while predisposing to thrombosis in vivo. In fact, approximately 30% of APA patients will experience thrombosis. A panel of assays is necessary to detect APAs as no single test presently available is sufficient.

Diagnosis of APS is made by clinico-pathologic evaluation. In addition to clinical criteria such as vascular thrombosis or pregnancy morbidity, repeated laboratory testing of APA is required for the diagnosis because of transient low level increase of APA in many clinical conditions including infection. The laboratory criteria include positive testing for one of the following on 2 or more occasions, at least 12 weeks apart: 1. lupus anticoagulant; 2. anticardiolipin antibodies (IgG or IgM) in medium or high titer; 3. B2GP1 antibodies (IgG or IgM).

### Lupus anticoagulant (LA) testing:

Based upon consensus criteria from the International Society for Thrombosis and Haemostasis (ISTH), confirmation of a LA requires that the following criteria are met:

- Performing two or more phospholipid-dependent clotting tests and demonstrating prolongation of at least one test (i.e. aPTT or dilute Russell Viper Venom Test (dRVVT))
- Evidence for inhibitory activity shown by the effect of patient plasma on normal pooled plasma. (i.e. positive mixing study)
- Demonstration of phospholipid-dependence of the inhibitor on a confirmatory test shown by shortening of the clotting

time with the addition of more phospholipid.

- Exclusion of a co-existing specific factor inhibitor, particularly factor VIII or an anticoagulant drug such as heparin or direct thrombin inhibitor (DTI).

### Anticardiolipin antibody (ACA) IgG, IgM or IgA, and B2GP1 antibody IgG or IgM testing:

ACAs recognize a complex of cardiolipin, a naturally found phospholipid, bound to a protein called B2GP1. Complexes of anionic phospholipids and endogenous plasma proteins provide more than one epitope recognized by natural autoantibodies. An enzyme-linked immunosorbent assay (ELISA) is performed for APA testing. Because the antigen target of ACAs is B2GP1 bound to cardiolipin, B2GP1 antibodies are considered to be more specific than ACA assays.

### Clinical Indications for Testing

Suspicion for APS in patients with an elevated aPTT, unexplained thrombocytopenia, or a history of arterial and venous thrombosis and/or obstetric complications.

### Interpretation

#### Lupus Anticoagulant

Tests for LA are interpreted as positive, indeterminate or negative. A narrative interpretation is issued for each patient panel.

**Positive:** Panel of tests meets all four diagnostic criteria. If one screening test, one mixing test and one confirmatory test are positive and there is no evidence for a factor inhibitor or anticoagulant drug effect, the diagnostic criteria for LA are fulfilled.

**Indeterminate:** Fewer than four diagnostic criteria are met. If clinical suspicion exists, the patient should be retested at a later date.

**Negative:** None of four diagnostic criteria is met.

#### Anticardiolipin Antibodies and B2GP1 Antibodies

Tests for ACA and B2GP1 are interpreted as positive, equivocal or negative. *Reference range of each test in the diagnostic panel is shown in Table 1.*

## Methodology

Laboratory testing for LA consists of a panel of assays (at least two assays on different principles in each criterion) specifically performed together to maximize diagnostic potential.

See the *LA Diagnostic Algorithm* located on the back page.

| Test Category         | Tests Performed  |
|-----------------------|--|
| Screening Tests       | aPTT, aPTT screen, dRVVT screen, Hexagonal PL screen                     |
| Mixing Studies        | Mixing Study aPTT (immediate and delayed), dRVVT mix                     |
| PL Confirmatory Tests | dRVVT confirm ratio, hexagonal PL confirm, platelet neutralization (PNP) |

**Screening Tests:** Four screening tests are performed: the standard laboratory automated aPTT, a more APA-sensitive manual aPTT screen reagent (which contains a different phospholipid composition), the dilute Russell's viper venom test (dRVVT), a clot-based assay that uses snake venom to activate Factor X directly, and the hexagonal PL screen, which uses a very dilute aPTT reagent to increase sensitivity to phospholipids.

**Mixing Studies:** Patient plasma and normal control plasma are mixed 1:1 and an aPTT and dRVVT test is performed on the mixed sample. In the presence of an inhibitor in the patient's plasma, the normal plasma also is affected, and the clotting time will not correct into the normal range. However, if the initial prolonged clotting time was due to a factor deficiency in the patient's plasma, the normal plasma corrects this deficiency and the resultant clotting time will be normal. The aPTT mixing study also includes a one-hour incubation step to check for more slow-acting specific factor inhibitors.

**PL Confirmatory Tests:** Several tests are used to confirm the phospholipid-dependence of an inhibitor.

- The dRVVT confirm ratio is performed by adding PL to plasma and repeating the dRVVT assay. The ratio is calculated by the dRVVT screen/dRVVT confirm.
- The hexagonal phase phospholipid test (STAclot) confirm is performed by adding hexagonal PL to plasma and repeating the hexagonal PL screen. The Delta is calculated by the hexagonal PL screen — the hexagonal PL confirm

- The platelet neutralization procedure (PNP) uses phospholipid-containing platelet membranes to neutralize the aPTT-prolonging effects of an LA. A PNP test is positive when the prolonged aPTT is shortened by the addition of platelet lysate.

**Exclusion Assays:** The presence of other inhibitors must be excluded to confirm the presence of an APA. These include drugs (heparin, DTIs) and specific factor inhibitors (factor VIII is the most common). Tests for each of these are included in the panel, as required per the LA algorithm.

Specific antibodies against cardiolipin and B2GP1 are measured by solid-phase ELISA assay.

## Limitations of the Assays

LAs are heterogeneous in terms of antigenic recognition, and aPTT reagents are variable in terms of phospholipid composition. Thus, variability in detection of LAs may exist between individual reagents, between different panel tests, and/or between laboratories.

Consequently, a normal aPTT cannot definitively exclude the presence of a LA; therefore, if clinical suspicion is high, the full panel may be performed.

Both ACA and B2GP1 APA assays are recommended because using one B2GP1 antibody assay can miss some cases of APA.

## References

1. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. *J. Thromb Haemost.* 2009; 7:1737.
2. Kottke-Marchant K. *An Algorithmic Approach to Hemostasis Testing.* CAP Press (2008).
3. Miyakis S, Lockshin MD, Atsumi T et al. International consensus statement on an update of the classification criteria for definite antiphospholipid antibody syndrome (APS). *J. Thromb Haemost.* 2006; 4:295.
4. Moffat KA, Ledford-Kraemer MR, Plumhoff EA et al. Are laboratories following published recommendations for lupus anticoagulant testing? An international evaluation of practices. *Thromb Haemost.* 2009;101:178.
5. Hoppensteadt D, Walenga J. The relationship between the antiphospholipid syndrome and heparin-induced thrombocytopenia. *Hematol Oncol Clin N Am* 2008;22:1.

**Table 1. Reference Range of Each Test in the Lupus Anticoagulant Diagnostic Interpretive Panel**

| Test Name                          | Reference Range   |
|------------------------------------|---|
| aPTT                               | 23.0 – 32.4 sec   |
| PT/INR                             | 8.4 – 13.0 sec / 0.8 – 1.2  |
| TT                                 | <18.6 sec   |
| Mixing study, incubated aPTT       | Negative  |
| Hexagonal Phase PL test            | Screen: 48.9 – 70.2 sec, Delta: < 9.0   |
| DRVVT                              | Screen: 32.7 – 46.7 sec, 1:1 Mix 32.7 – 46.7 sec, Confirm Ratio: < 1.21   |
| PNP                                | Negative  |
| ACA IgG:                           | Negative: < 10 GPL, Equivocal: 10 – 40 GPL, High Positive: > 40 GPL<br>IgM: Negative: < 12 MPL, Equivocal: 12 – 40 MPL, High Positive: > 40 MPL<br>IgA: Negative: < 12 APL, Equivocal: 12 – 40 APL, High Positive: > 40 APL |
| B2GP1 Autoabs                      | IgG: < 20 Units, IgM: < 20 Units  |
| Heparin Assay/Factor Xa inhibition | < 0.10 IU/mL  |

### Test Overview

|                                |   |
|--------------------------------|---|
| Test Name                      | Lupus Anticoagulant Diagnostic Interpretive Panel   |
| Ordering Mnemonic              | LUPUSP  |
| Reference Range                | See Table 1   |
| Specimen Requirements          | 1. Testing Volume/Size: 1 mL; Type: Serum; Tube/Container: SST (Gold); and<br>2. Testing Volume/Size: 5 mL; Type: Plasma; Tube/Container: Sodium citrate (Lt. Blue).<br><i>Please indicate each tube as serum or plasma</i>   |
| Specimen Collection & Handling | Collection of blood by routine venipuncture in a 3.5ml light blue top tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant.<br><i>Please refer to "Criteria for rejection and special handling of coagulation specimens".</i>   |
| Patient Preparation            | Discontinue heparin therapy for 2 days prior to collection. If tests are abnormal, the following tests may be ordered and billed: Factor II (85210), Factor V (85220), Factor X (85260), Factor VIII (85247), von Willebrand Factor Antigen (85246), Ristocetin Co-factor (85245), Factor IX Assay (85250), Factor XI Assay (85270), Factor XII Assay (85280), Heparin fXa inhibition (85520), Fibrinogen and Bethesda Assay. |
| Test Ordering Information      | 3.2% sodium citrate is the preferred anticoagulant recommended by CLSI.   |
| Billing Code                   | 24  |
| CPT Code                       | 85390; 85597; 85610; 85613(x2); 85730(x3); 85732(x3); 86146(x2); 86147(x3), 85670   |

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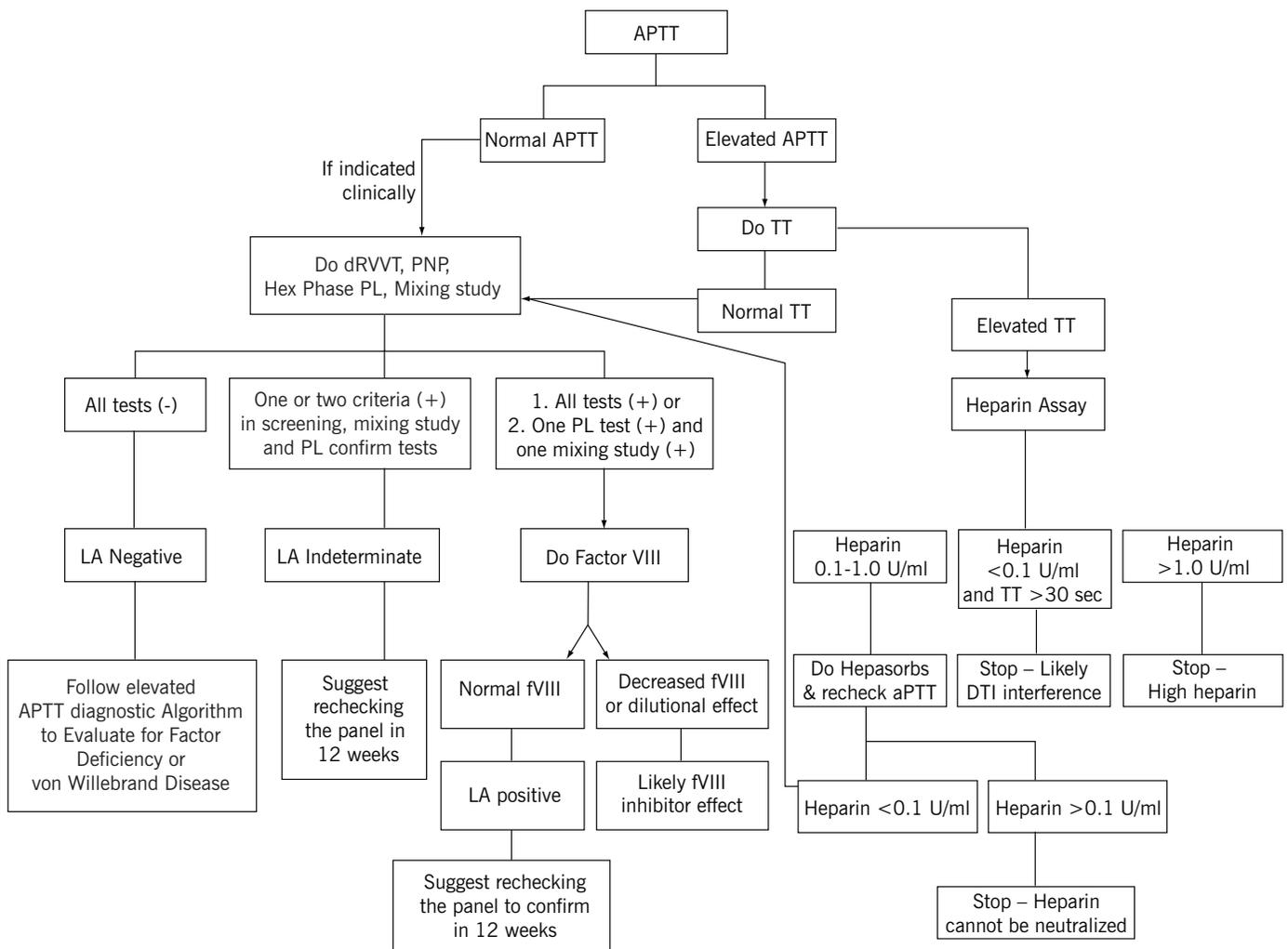
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Lupus Anticoagulant Diagnostic Algorithm



**Abbreviations:** APTT - activated partial thromboplastin time, dRVVT - dilute Russell's viper venom test, fVIII - factor VIII, LA - lupus anticoagulant, PL - phospholipid, PNP - platelet neutralization procedure, TT - Thrombin time