Lupus anticoagulant
Pocket card

Issue number 5 2012
The antiphospholipid syndrome (APS) is diagnosed in patients with recurrent thromboembolic events and/or pregnancy loss in the presence of persistent laboratory evidence for antiphospholipid antibodies.

Laboratory criteria include:
- Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart and detected according to the ISTH SSC guidelines.
Lupus anticoagulant (LA)

• Antibodies directed specifically against the phospholipid moiety of the prothrombinase complex

• **In vitro: anticoagulant;** LA inhibits phospholipid-dependent coagulation reactions:
  • Activation of Factor X to Factor Xa by Factor VIIa-tissue factor
  • Activation of Factor X by Factor IXa in the presence of Factor VIIIa and calcium ions
  • Activation of prothrombin by Factor Xa in the presence of Factor Va and calcium ions

• **In vivo: prothrombotic;** presence of LA is associated with:
  • Increased arterial and venous thrombosis
  • Stroke
  • Transient ischaemic attacks
  • Recurrent spontaneous abortions
  • Acquired thrombophilia

• LA has the strongest link of all aPLs to thrombotic risk/pregnancy complications

• Accurate diagnosis is essential for risk assessment and long-term patient management with anticoagulant therapy

• LA is identified by a systematic, laboratory-based approach that includes:
  1. Prolongation of a phospholipid-dependent screening assay
  2. Demonstration of inhibitory activity by mixing studies with healthy pooled plasma
  3. Confirmatory assay showing that the inhibitory activity is phospholipid dependent

• No single test is sensitive for all LA
Effect of lupus anticoagulant on phospholipid-dependent assays

PL, phospholipid; Ca\(^{2+}\), calcium ions
Coagulation cascade showing phospholipid-dependent steps and effects on activated partial thromboplastin time (aPTT), the dilute Russell’s viper venom time (dRVVT) and the prothrombin time (PT) by the presence of lupus anticoagulant in vitro.

Fig. 1
Appropriateness for lupus anticoagulant (LA) testing (ISTH guidelines)³

Testing for LA should be limited to patients who have a significant probability of having the antiphospholipid syndrome or who have unexplained prolonged activated partial thromboplastin time (aPTT). Appropriateness to search for LA can be graded according to clinical characteristics:

- **Low**
  - Venous (VTE) or arterial thromboembolism in elderly patients

- **Moderate**
  - Accidentally found prolonged aPTT in asymptomatic subjects
  - Recurrent spontaneous early pregnancy loss
  - Provoked VTE in young patients

- **High**
  - Unprovoked VTE and (unexplained) arterial thrombosis in young patients (<50 years of age)
  - Thrombosis at unusual sites
  - Late pregnancy loss
  - Any thrombosis or pregnancy morbidity in patients with autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, autoimmune thrombocytopenia, autoimmune haemolytic anaemia)
ISTH recommendations for the optimal laboratory detection of lupus anticoagulant (LA)³

A Blood collection

1. Blood collection before the start of any anticoagulant drug or a sufficient period after its discontinuation
2. Fresh venous blood in 0.109 M sodium citrate (ratio 9:1)
3. Double centrifugation
4. Quickly frozen plasma is required if LA detection is postponed
5. Frozen plasma must be thawed at 37°C

B Choice of test

1. Two tests based on different principles
2. Dilute Russell’s viper venom time (dRVVT) should be the first test considered
3. The second test should be a sensitive aPTT (low phospholipids and silica as activator)
4. LA should be considered as positive if one of the two tests gives a positive result

C Mixing test

1. Pooled normal plasma (PNP) for mixing studies should ideally be prepared in-house. Adequate commercial lyophilized or frozen PNP can alternatively be used
2. A 1:1 proportion of patient-to-PNP shall be used, without pre-incubation within 30 min
3. LA cannot be conclusively determined if the thrombin time (TT) of the test plasma is significantly prolonged

D Confirmatory test

1. Confirmatory test(s) must be performed by increasing the concentration of phospholipids (PL) of the screening test(s)
2. Bilayer or hexagonal (II) phase PL should be used to increase the concentration of PL

E Expression of results

1. Results should be expressed as ratio of patient-to-PNP for all procedures (screening, mixing and confirmatory)

F Transmission of results

1. A report with an explanation of the results should be given

Table 1

Once a patient has been identified as positive for LA, it is imperative that testing be repeated on a second occasion >12 weeks after initial testing.
**ISTH cut-off values for lupus anticoagulant (LA) detection**

**Screening test**
How should this be determined?
1. Perform testing on plasma from healthy donors
2. Take the cut-off as the value above the 99th percentile of the distribution

Interpretation:
1. Results of screening tests are potentially suggestive of LA when their clotting times are longer than the local cut-off value

**Mixing test**
How should this be determined?
1. Perform testing on plasma from healthy donors mixed with the pooled normal plasma (PNP) at 1:1 proportion. Testing should be performed without pre-incubation within 30 min
2. Take the cut-off as the value above the 99th percentile of the distribution
3. Alternatively, the cut-off may be the value of the index of circulating anticoagulant (ICA) defined according to the equation: $ICA = \[(b-c)/a\] \times 100$ where $a$, $b$ and $c$ are the clotting times of the patient plasma, mixture and normal plasma, respectively

Interpretation:
1. Results of mixing tests are suggestive of LA when their clotting times are longer than the local cut-off value, or when the ICA is greater than the local cut-off value

*Testing described must be performed with the local reagent/instrument combination on plasma from at least 40 adult healthy donors <50 years of age. Do not use cut-off values established elsewhere even if they refer to the same method and coagulometer.*
**Confirmatory test**

How should this be determined?

1. Perform testing on plasma from healthy donors at low (screen) and high (confirm) phospholipid concentrations
2. Take the cut-off as the value corresponding to the mean of the individual % corrections calculated as defined by the equation: 
   \[ \frac{\text{screen-confirm}}{\text{screen}} \times 100 \]

Interpretation:
1. Results are confirmatory of LA if the % correction is above the local cut-off value

* The clotting time of the confirmatory test in LA positive samples is not always shortened to within the normal range of controls. To avoid false-negative results, the ISTH recommends confirmatory tests to be performed in all the normal controls and to use the mean of obtained clotting times to calculate the percentage of shortening. This percentage can be used as a cut-off value.
Lupus anticoagulant (LA) detection in patients on long-term vitamin K antagonists (VKA) (ISTH guidelines)³

1. The interpretation of results is difficult because of the prolonged basal clotting time. To avoid misinterpretation, it is recommended:
   • To perform laboratory procedures 1 to 2 weeks after discontinuation of treatment or when the international normalised ratio (INR) is less than 1.5
   • Where bridging vitamin K antagonist (VKA) discontinuation with low molecular weight heparin (LMWH), the last dose of LMWH should be administered more than 12 h before blood is drawn for LA testing

2. Alternatively, if the INR is between 1.5 and $<3.0$, a 1:1 dilution of patient plasma and pooled normal plasma (PNP) can be considered. Note: interpretation of results may still be difficult and that the LA titre will be diluted 2-fold

3. Detection procedures by textarin and/or Ecarin clotting times or integrated tests (i.e. % correction for activated partial thromboplastin time [aPTT] and dilute Russell’s viper venom time [dRVVT] at low and high phospholipid concentration) are not currently recommended as they require further critical evaluation
### Effects of lupus anticoagulant (LA) and anticoagulant therapies on clinical laboratory assays

<table>
<thead>
<tr>
<th>Test</th>
<th>LA</th>
<th>Therapeutic UFH</th>
<th>Therapeutic LMWH</th>
<th>Therapeutic fondaparinux</th>
<th>Warfarin</th>
<th>Direct thrombin inhibitors</th>
<th>Direct factor Xa inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>↔/↑</td>
<td>↔/Mild ↑ ↔</td>
<td>↔</td>
<td>↑</td>
<td>Variable</td>
<td>↑ Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>aPTT</td>
<td>↑</td>
<td>↑</td>
<td>Mild ↑</td>
<td>Minimal</td>
<td>↔/Mild↑</td>
<td>↑ Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>TCT</td>
<td>↔</td>
<td>↑</td>
<td>Variable↑ ↔</td>
<td>↔</td>
<td>↔↑ Variable</td>
<td>↑ Variable</td>
<td>↑</td>
</tr>
<tr>
<td>dRVVT (screening)</td>
<td>↑</td>
<td>↑</td>
<td>Variable↑ ↔</td>
<td>↔</td>
<td>↑↑ Variable</td>
<td>↑ Variable</td>
<td>↑&quot;</td>
</tr>
<tr>
<td>Anti-factor Xa</td>
<td>Not present</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Not present</td>
<td>Not present</td>
<td>↑</td>
</tr>
</tbody>
</table>


- **a** Direct thrombin inhibitors include the parenteral agents lepirudin, argatroban and bivalirudin, and the oral agent dabigatran etexilate.
- **b** Direct factor Xa inhibitors include rivaroxaban, apixaban and edoxaban; relatively limited data are available on the latter two agents.
- **c** Results are for lepirudin, argatroban and bivalirudin; no data are available for dabigatran etexilate.
- **d** Results are for rivaroxaban only.

- **aPTT**, activated partial thromboplastin time; **dRVVT**, dilute Russell’s viper venom time; **LMWH**, low molecular weight heparin; **PT**, prothrombin time; **TCT**, thrombin clotting time; **UFH**, unfractionated heparin; ↔ normal; ↑ prolonged/elevated.

Overview of ISTH recommendations for optimal laboratory detection of lupus anticoagulant (LA)²

<table>
<thead>
<tr>
<th>Collection of blood samples</th>
<th>Not recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh venous blood 0.109 M sodium citrate (ratio 9:1)</td>
<td>Plasma filtration</td>
</tr>
<tr>
<td>Avoid testing if on anticoagulants</td>
<td></td>
</tr>
<tr>
<td>Double centrifugation</td>
<td></td>
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<tr>
<td>Quick freeze plasma; thaw at 37° C</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Screening test</th>
<th>aPTT using kaolin activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two tests using different principles:</td>
<td>Ellagic activation</td>
</tr>
<tr>
<td>1. Dilute Russell’s viper venom time (dRVVT)*</td>
<td>Dilute prothrombin time (dPT)</td>
</tr>
<tr>
<td>2. Sensitive aPTT (low phospholipids [PL] and silica as activator)</td>
<td>Kaolin clotting time</td>
</tr>
<tr>
<td>3. Cut-off value 99th percentile</td>
<td>Ecarin and textarin clotting times</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mixing test**</th>
<th>Frozen/thawed platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled normal plasma (PNP) 1:1</td>
<td></td>
</tr>
<tr>
<td>No pre-incubation</td>
<td></td>
</tr>
<tr>
<td>Cut-off value 99th percentile</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Confirmatory test</th>
<th>Use of terms such as borderline or dubious LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase concentration of phospholipids in screening test</td>
<td></td>
</tr>
<tr>
<td>Bilayer or hexagonal (II) phase to increase the concentration of PL</td>
<td></td>
</tr>
<tr>
<td>Cut-off value by % correction</td>
<td></td>
</tr>
<tr>
<td>[(screen-confirm)/screen] × 100</td>
<td></td>
</tr>
<tr>
<td>or LA ratio (screen/confirm)</td>
<td></td>
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</table>

| Reporting | |
|------------||
| Report results as ratio of patient-to-PNP | |
| Give interpretive comment indicating whether results are compatible with presence/absence | |
| of LA | |

Once a patient has been identified as positive for LA, it is imperative that testing be repeated on a second occasion >12 weeks after the initial testing.

Table 3: adapted from Pengo V. et al. (2009). J Thromb Haemost.

* dRVVT may also be prolonged by deficiency or abnormality of factors II, V, X, or fibrinogen, but this does not correct on addition of PL in confirmatory test.

** The coagulation time of a mixing test could also be prolonged in the presence of heparin or inhibitors to coagulation factors. The thrombin time (TT) performed on test plasma or the clinical history of bleeding will help to identify heparin or specific inhibitors to clotting factors, respectively.

aPTT, activated partial thromboplastin time.
References

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