PRINCIPLE
This is a non-specific test of the intrinsic system. Taken in conjunction with a normal prothrombin time, it is the most useful screening test for detecting deficiencies of factors VIII, IX, XI, and XII.

The APTT will also be prolonged in any deficiency involving the common pathways (deficiencies of factors V, X, II and, to a lesser extent, fibrinogen,) and in the presence of inhibitors. The presence of some therapeutic inhibitors of coagulation such as heparin will also prolong APTT. It is important to exclude the possibility that such treatments have been employed in the initial investigation of prolonged APTTs.

The APTT is prolonged in the presence of prekallikrein (PKK) or high-molecular-weight kininogen (HMWK) deficiency unless the test is performed using a reagent that contains ellagic acid as the activator. In that case, the APTT will be normal, even in the complete absence of these factors.

The pathway measured by the APTT is shown in Figure 13.2.

REAGENTS
- APTT reagent
- 25mM calcium chloride

METHOD
1. Place tube containing calcium chloride at 37°C for five minutes prior to use.
2. Pipette 0.1 ml of APTT reagent into two glass clotting tubes at 37°C.
3. Pipette 0.1 ml control plasma into first tube.
5. Add 0.1 ml control plasma to second tube. Mix.
6 After recommended incubation time*, add 0.1 ml calcium chloride to each tube in succession, starting a new stopwatch for each tube. Mix. Time clot formation.

For manual technique, perform all tests in duplicate. Duplicate clotting times should not differ by more than 10%. For automated tests with a between assay coefficient of variation of less than 5%, single replicates will normally be acceptable, provided prolonged results are checked.

*The reagent manufacturer’s recommendation should be followed. This is normally in the range of two to five minutes. It is important that the incubation is timed exactly, since deviations from this will normally affect the results, with longer incubations giving shorter clotting times for any particular reagent.

**INTERPRETATION**

A normal range should be established locally.

A long APTT with a normal PT indicates a possible deficiency of factor VIII, IX, XI, XII, high-molecular-weight kininogen, prekallikrein, or the presence of an inhibitor. In cases of a long APTT, an equal mixture of normal and test plasma should be tested (i.e., a mixture of 1 part test and 1 part normal plasma, called a 50:50 mix, below). If the APTT corrects by more than 50% of the difference between the clotting times of the normal and test plasma, a factor deficiency is indicated. Poor correction suggests an inhibitor, possibly to one of the clotting factors in the system or of the non-specific type, such as lupus anticoagulant (see Section 25).

**Figure 13.1. An example of interpretation of APTT**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT control</td>
<td>35 seconds</td>
</tr>
<tr>
<td>Test</td>
<td>60 seconds</td>
</tr>
<tr>
<td>If 50:50 mix</td>
<td>42 seconds (this is a good correction, so there is probably a factor deficiency)</td>
</tr>
<tr>
<td>If 50:50 mix</td>
<td>52 seconds (this is a poor correction, so an inhibitor is probably present)</td>
</tr>
</tbody>
</table>
NOTES

• Many suitable reagents are commercially available. These include materials with different sensitivities. Notes on reagent selection are included in Section 9.

• As for PT, clotting times can be influenced by the use of a coagulometer.

• Within test plasmas, high levels of one clotting factor can compensate for lower levels of other factors. For example, a markedly raised FVIII during acute phase reaction can lead to a normal APTT in the presence of reductions of FIX or FXI, which could be clinically important. If a patient has the appropriate personal or family history suggestive of a bleeding disorder, fuller investigation, including specific factor assays, may be justified in the presence of a normal APTT, particularly if the result lies in the upper part of the reference range.

• The concentration of phospholipid varies markedly between reagents. This is one reason why reagents vary markedly in their sensitivity to the presence of lupus anticoagulants. If a lupus-sensitive reagent is used for the initial APTT, it is useful to perform a second APTT using a reagent such as Actin FS (Dade Behring, Marburg, Germany), which has a very high phospholipid concentration (Kitchen et al. 1999). If the prolongation with the first reagent is caused by lupus anticoagulant, then the second APTT is almost always normal, since very few lupus anticoagulants prolong APTT when Actin FS is used.

INVESTIGATION OF ISOLATED PROLONGED APTT

For patients with a normal prothrombin time and prolonged APTT, the normal sequence of investigation to follow is:

1 Determine thrombin time (see Section 15). If it is normal, proceed to steps 2 and 3. If thrombin time is prolonged, repeat in the presence of protamine sulphate (see Section 16). If the thrombin time is corrected to normal, this suggests that heparin is present, and further tests below are not required. If the patient is not known to be receiving heparin of any kind, a repeat sample should be requested.

2 Determine APTT on mixtures of normal and patient plasma (see Section 14) using a 1:1 (50%) mixture of normal:patient. Failure of the 50% mixture to correct the APTT to normal indicates presence of an inhibitor.

3 Determine APTT with a second reagent that contains high concentration phospholipids, such as Actin FS (Dade Behring). If the initial APTT is clearly prolonged (by at least three seconds over the upper limit of normal in use) and the Actin FS is normal, then lupus anticoagulant is the
likely cause. This can be confirmed later by specific tests such as dilute Russell’s viper venom time (DRVVT; see Section 38), although this is not normally necessary in the absence of any requirement to investigate possible lupus anticoagulant (LAC) as a risk factor for thrombosis. Very rarely, deficiency of prekallikrein is the other possible cause of normal APTT with Actin FS and marked prolongation of APTT with a reagent that uses silica or kaolin as activator. Like most cases of LAC, this is not associated with any bleeding risk. Therefore, again, confirmation may not be required.

4 When initial APTT is clearly prolonged (three or more seconds) and Actin FS APTT is normal, there is no need to perform factor assays.

5 If both APTTs are prolonged, perform FVIII:C, FIX, and FXI assays as required (see Section 23). A FXII assay can be performed if required, since deficiency is relatively common and detection of this can then explain the prolongation of APTT. This is not necessary to exclude the presence of a bleeding disorder, since deficiency of FXII is not associated with increased bleeding risk.

6 Reagents such as Actin FS, which employ ellagic acid as the contact activator, are associated with normal results in the presence of even severe deficiency of prekallikrein.

Where necessary, steps 1 to 3 can be performed concurrently to save time.

NOTES

- A normal APTT with Actin FS, combined with an initial prolonged APTT, normally excludes the presence of FVIII, FIX, or FXI deficiency, and in this case there is no need for factor assays.
- Rarely, a normal APTT with any reagent can occur when FIX or FXI are mildly reduced (30-50 U/dl) and FVIII is markedly elevated.
- APTT with Actin FS is frequently normal when FXII is reduced in the range of 20 U/dl-50 U/dl and APTT with kaolin or silica-based activation is mildly elevated. This defect has no clinical relevance.
- A few powerful lupus anticoagulants prolong APTT with Actin FS.
- Specific antibodies to FVIII (or FIX or FXI) prolong APTT, irrespective of reagent.

For a full discussion of issues related to determination of APTT, see CLSI (2008).
**Figure 13.2. The pathway measured by APTT**

Contact Activation involving Prekallikrein, High Molecular Weight Kininogen and negatively charged surface

- Factor XII → Factor XIIa
- Factor XI → Factor Xla with Ca++/PL
- Factor IX → Factor IXa with Ca++/PL/Factor VIII
- Factor X → Factor Xa with Ca++/PL/Factor V
- Factor II → Factor IIa
- Fibrinogen → Fibrin

PL = Phospholipid → Activation

**REFERENCES**
