PRINCIPLE
This test reflects the overall efficiency of the extrinsic system. It is sensitive to changes in factors V, VII, and X, and less so to factor II (prothrombin). It is also unsuitable for detecting minor changes in fibrinogen level, but may be abnormal if the fibrinogen level is very low or if an inhibitor is present. The sensitivity of the test is influenced by the reagent and technique used, and it is important to establish a reference range locally.

The pathway measured by the prothrombin time is shown in Figure 12.1, on page 39. The PT reagent, often termed thromboplastin, contains tissue factor and phospholipids.

Many suitable reagents are commercially available. Notes on reagent selection are included in Section 9.

REAGENTS
- Thromboplastin (this may contain calcium chloride)
- 25mM calcium chloride (required only if thromboplastin reagent does not contain calcium)

METHOD: MANUAL
1. To the first two tubes, add 0.1 ml normal plasma and warm to 37°C for 2 minutes.
2. Add 0.2 ml pre-warmed (to 37°C) thromboplastin reagent (if calcium is present in the reagent).
3. Start stopwatch, mix, and record clotting times.
4. Repeat for each test sample.
5. Report patient’s clotting time in seconds.
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 CV of less than 5%, single tests will normally be acceptable, provided prolonged results are checked.

**NOTES**

- If thromboplastin reagent does not contain calcium, the test procedure is 0.1 ml plasma, 0.1 ml thromboplastin, and clot with 0.1 ml pre-warmed 25mM calcium chloride.

- Activation of FIX by tissue factor: FVII occurs *in vivo*. Under the conditions of most PT tests, FX is so strongly activated that the assay is insensitive to deficiency of FIX or FVIII.

- Thromboplastin/calcium chloride should be pre-warmed for 5 to 30 minutes prior to use.

- Clotting times are normally influenced by the use of different coagulometers, depending on how and when the end point is detected. This further emphasizes the importance of establishing normal ranges for the method currently in use in the laboratory.

- In the presence of mild deficiencies of factor II, V, VII, or X, the degree of prolongation may be minimal. In the case of FII deficiency, the PT may be within the normal range.

- Some PT reagents can be affected by the presence of lupus anticoagulants/anti-phospholipid antibodies, and some rare types of antibody may prolong the PT without any prolongation of APTT. Reagents with lower phospholipid concentrations are more likely to be affected, including some reagents that are constructed by lipidating recombinant tissue factor.

- The presence of activated FVII, either following therapy with recombinant VIIa or when native FVII has been activated, can shorten the PT. The effect is dependent on the tissue factor reagent used. Reagents containing bovine tissue factor are particularly susceptible to this effect (Kitchen et al. 1992). Blood samples should not be stored at 2°C–8°C prior to determination of PT, since cold activation of FVII may occur.

- Whole blood for PT determination may be stable for at least 24 hours, depending on the reagent used (Baglin and Luddington 1997).

- PTs determined with reagents containing human tissue factor may be different from those obtained with reagents containing tissue factor from other species, such as rabbit. In such cases, the result obtained with human tissue factor reagents may be more indicative of bleeding risk.

- For a full discussion of issues related to determination of PT, see CLSI (2008).
Fig 12.1. Pathway measured by the prothrombin time test

Ca\(^{++}\)/PL/Tissue Factor + Factor VII

Factor X → Factor Xa with Ca\(^{++}\)/PL/Factor V

Prothrombin (Factor II) → Thrombin (Factor IIa)

Fibrinogen → Fibrin

PL = Phospholipid → Activation

REFERENCES

