The assays of factors II, V, VII, and X can be performed using a one-stage assay based on the prothrombin time. Essentially, the assay consists of comparing the ability of dilutions of a standard or reference plasma and test plasma to correct the prothrombin time of a plasma known to be totally deficient in the clotting factor being measured. In a factor V assay, for example (described below), the plasma is deficient in factor V but contains normal amounts of factors II, VII, X, and fibrinogen. Clotting factors II, VII, and X may be assayed in a similar way, substituting the appropriate deficient plasma for FV-deficient plasma in the example given below, and using an appropriate reference plasma with a known concentration of the factor being assayed.

**REAGENTS**

- FV-deficient plasma
  This may be congenitally deficient or artificially deficient in factor V (aged plasma)
- Owren’s buffered saline
  OBS or glyoxaline buffer (see Section 9)
- Platelet-poor citrated plasma: test and standard
  For the standard, use a 20-donor normal plasma pool (kept at -70°C or below) or a commercial reference or standard plasma.
- Thomboplastin/calcium (as used in PT tests)

**METHOD**

1. For both test and standard plasmas, prepare dilutions in plastic tubes, as shown in Figure 22.1 below.

**Figure 22.1. Preparation of test and standard plasma dilutions**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Plasma (ml)</th>
<th>OBS (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/5</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>1/10</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>1/20</td>
<td>0.5 (1/10)</td>
<td>0.5</td>
</tr>
<tr>
<td>1/40</td>
<td>0.5 (1/20)</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Note: Mix the 1/10 dilution well before using it to prepare the 1/20 dilution. Mix the 1/20 dilution well before using it to prepare the 1/40 dilution. Plasma dilutions should be tested immediately after preparation. If room temperature exceeds 25°C, it may be necessary to keep dilutions on wet ice prior to analysis.

2 Test each dilution of reference or standard plasma as follows:
   i. Pipette 0.1 ml of each dilution into a 75 × 10 mm glass tube.
   ii. Add 0.1 ml factor V deficient plasma.
   iii. Warm to 37°C for 2 minutes.
   iv. Add 0.2 ml pre-warmed thromboplastin/calcium reagent.
   v. Start stopwatch and mix.

Note: If the thromboplastin reagent does not contain calcium, 0.1 ml of thromboplastin is added to the mixture of dilution and deficient plasma. After a 1–2 minute delay for warming to 37°C, the mixture is clotted with 0.1 ml pre-warmed (to 37°C) calcium.

3 Record clotting time.

4 Repeat for dilutions of test plasma.

For test plasmas expected to be normal, test 1/10, 1/20, and 1/40 dilutions. For test plasmas expected to have reduced levels, test 1/5, 1/10, and 1/20 dilutions.

A “blank” should also be tested as follows:
   • 0.1 ml OBS
   • 0.1 ml factor V-deficient plasma
   • 0.2 ml thromboplastin/calcium reagent

The clotting time of the “blank” reflects the quality of the deficient plasma and should be equivalent to less than 1%.

RESULTS

Plot clotting times of control and test plasmas against concentration of FV on 3 cycle × 2 cycle logarithmic paper. An example of such a graph (for a FVIII assay) is shown in Section 23. The 1/10 dilution is arbitrarily assigned a value of 100%, thus the 1/5 dilution is equivalent to 200%, etc. Alternatively, plot concentration on a logarithmic scale and clotting time on a linear scale.

The relative amount of FV in the patient’s plasma compared with normal plasma or standard reference material is extrapolated from the graphs. An example of this is shown in the section on APTT-based assays (Figure 23.1).
The clotting time equivalent to 100% test (the place where the test line passes through the 100% activity) is read from the standard line (therefore, the concentration of standard that could give that particular clotting time). This gives the concentration of the test in percentage of standard. This percentage is multiplied by the concentration of clotting factor in the standard (in IU/dl) to give the concentration in the test (in IU/dl).

NOTES

- The normal range for each of these clotting factors should be determined locally but often has a lower limit of 50–70 IU/dl for FV, FVII, and FX. The lower limit of normality for FII is higher. In one study, normal subjects from families with a history of FII deficiency, as well as other unrelated normal subjects, had FII levels in the range of 84–130 IU/dl (Girolami et al. 1998). A different centre has reported a reference range of 84–132 IU/dl (Bolton-Maggs et al. 2004).

- Individuals with a reduced level of FV should also have a FVIII assay performed to exclude combined FV and FVIII deficiency.

- In some cases of FVII deficiency, there may be a discrepancy between the levels of FVII:C obtained, depending on the source of thromboplastin. The use of human thromboplastin is therefore advisable on the basis that the results are more likely to reflect the in vivo activity. See Bolton-Maggs et al. (2004) for a review. In some rare cases, the result may be very low if rabbit thromboplastin is used, but normal if the assay utilizes human thromboplastin. This may be a reason why some cases of apparent severe FVII deficiency do not have bleeding symptoms.

REFERENCES
