**PRINCIPLE**

Coagulation inhibitors affecting the APTT may be immediate-acting or time-dependent. Test plasma containing an immediate-acting inhibitor will, when mixed with normal plasma, show little or no correction of the clotting time. Time-dependent inhibitors, on the other hand, require a period of incubation with normal plasma before they can be detected.

Normal plasma and test plasma are incubated at 37°C for 1–2 hours, both separately and as a 50:50 mixture. The APTT is then determined on the normal plasma, test plasma, and incubated mixture, as well as on a mixture prepared from equal volumes of test and normal plasma after separate incubation (immediate mix). The degree of correction of the APTT of each mixture is compared.

Poor correction in the mixture prepared after separate incubation is suggestive of an immediate-acting inhibitor. Poor correction in the incubated mixture is suggestive of a time-dependent inhibitor.

**REAGENTS**

- Normal plasma: pool of 20 donors
- Test plasma
- Reagents for APTT

**METHOD**

1. Prepare three plastic tubes: A, B, and C.
2. Into A, place 0.5 ml normal plasma, into B, 0.5 ml test plasma, and into C, 0.2 ml each of normal and test plasma.
3. Incubate for 1–2 hours at 37°C.
4. Make a 50:50 mix from tubes A and B. This is tube D, which serves as an immediate mix.
5. Perform an APTT in duplicate on A, C, D, and B (in that order).
RESULTS AND INTERPRETATION

Figure 28.1. Example of a clotting factor inhibitor screen based on APTT

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Normal plasma</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>B: Test plasma</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>C: Test + normal (incubated after mixing together)</td>
<td>45</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>D: Test + normal (incubated separately before mixing)</td>
<td>45</td>
<td>48</td>
<td>70</td>
</tr>
</tbody>
</table>

Sample 1: a plasma with an intrinsic defect but no inhibitor
Sample 2: a plasma containing a time-dependent inhibitor
Sample 3: a plasma containing an immediate-acting inhibitor