PRINCIPLE
Reptilase is a snake venom obtained from Bothrops atrox. It is a thrombin-like enzyme and acts directly on fibrinogen to convert it to fibrin. It is not inhibited by antithrombin, so it is not affected by the presence of heparin. Therefore, it can be used to assess the rate of fibrinogen → fibrin conversion in the presence of heparin.

It is useful to check whether a prolonged thrombin time is caused by the presence of heparin in the sample. If thrombin time is prolonged and reptilase is normal, the most likely cause is the presence of heparin. In the presence of dysfibrinogenemia, the reptilase time may be more sensitive (i.e. more prolonged) than thrombin time.

REAGENTS
- Reptilase (Sigma Aldrich, Code V5375) dissolved at a concentration of 25 mg in 15 ml Owren’s buffer. This crude venom is hazardous, and care must be taken to avoid inhaling the powder. The operator should wear gloves and a mask while handling the crude venom. The stock solution should be stored deep-frozen at -70°C in 0.5 ml aliquots. It is stable for at least two years under these conditions.

  To prepare ready-to-use reagent, thaw and dilute stock reagent 1/10 in Owren’s buffer; aliquot and re-freeze at -70°C for further use. This ready-to-use reagent is stable under these conditions for at least three months.

  Ready-to-use frozen aliquots should be thawed in a 37°C water bath for at least three minutes. This is then stable for use for at least 12 hours at ambient temperatures of 20°C–25°C.

- Normal plasma: Pooled normal plasma prepared as described in Section 7. Thaw in a 37°C water bath for approximately three minutes.

METHOD
Perform all tests in duplicate.

1 Place sufficient 75 x 10 mm glass clotting tubes in a water bath at 37°C (two per patient, plus two for the control).
2 Pipette 0.3 ml plasma (control or patient) into warm clotting tubes.

3 Warm for one to two minutes.

4 Add 0.1 ml reptilase dilution and start stopwatch.

5 Tilt three times to mix, then three times every five seconds until clot formation.

6 Record clotting time.

7 The control time should be 15 to 18 seconds. (If shorter, adjust by further diluting the reptilase reagent with Owren’s buffer.)

8 If no clotting occurs, report as >90 seconds.

NORMAL RANGE
Patient’s time should be within three seconds of the control time. Control time should be reported with patient’s time.

INTERPRETATION

Figure 17.1. Interpretation of prolonged thrombin time

<table>
<thead>
<tr>
<th>Thrombin time</th>
<th>Reptilase time</th>
<th>Cause</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged</td>
<td>Equally prolonged</td>
<td>Hypo- or afibrinogenemia</td>
<td>Measure fibrinogen</td>
</tr>
<tr>
<td>Prolonged</td>
<td>Strongly prolonged</td>
<td>Dysfibrinogenemia</td>
<td>Congenital or acquired</td>
</tr>
<tr>
<td>Prolonged</td>
<td>Normal</td>
<td>Heparin</td>
<td></td>
</tr>
<tr>
<td>Prolonged</td>
<td>Slightly prolonged</td>
<td>Heparin with some hypo- or dysfibrinogenemia</td>
<td>Rare case of dysfibrinogenemia may give this pattern</td>
</tr>
<tr>
<td>Prolonged</td>
<td>Equally prolonged</td>
<td>Disseminated intravascular coagulation (DIC)</td>
<td>Measure D-dimers</td>
</tr>
</tbody>
</table>
NOTE

Reptilase reagents are available at a ready-to-use concentration from several commercial manufacturers. The advantage of these is that there is no need to handle the crude venom, with its health and safety issues. If using one of these, follow the manufacturer’s instructions for use. The normal range may be different to that described above, but interpretation of results is as listed in Figure 17.1. Where reptilase is an expensive reagent, the protamine neutralization/thrombin time method (Section 16) can be used to confirm the presence of heparin in the test sample.