Fibrinogen (Modified Clauss Assay)

**PRINCIPLE**

Dilutions of standard normal plasma with known fibrinogen content are prepared in glyoxaline buffer. The clotting time is measured after the addition of thrombin, and a graph is constructed.

The clotting time is proportional to the concentration of fibrinogen, and the 1/10 dilution is taken to represent the value in the standard preparation. The test plasma is diluted 1/10, and the result read from the standard line.

**REAGENTS**

- Standard or reference plasma with known fibrinogen concentration
- Thrombin 30 U/ml–100 U/ml (concentration may vary according to source).
- Imidazole buffer (glyoxaline) or Owren’s buffer pH 7.35

**METHOD**

1. Prepare 1/5, 1/10, 1/15, and 1/20 dilutions of standard plasma in imidazole buffer.
2. Pipette duplicate 0.2 ml volumes of each dilution into glass clotting tubes.
3. Warm to 37°C for two minutes.
4. Add 0.2 ml thrombin (30 U/ml–100 U/ml) and time the clot formation.
5. For manual techniques, test in duplicate. This is not necessary for most coagulometers when the test is automated.
6. Plot the mean clotting time versus fibrinogen concentration on log/log graph paper, taking the 1/10 dilution to represent the standard value.
7. Dilute the test plasma 1/10, determine the clotting time, and read the value off the graph.
The normal range should be established locally, but is usually close to 1.5 g/l–3.5 g/l.

For most Clauss techniques, the relationship between clotting time and fibrinogen concentration is linear over a limited range of clotting times, typically 10 to 25 seconds.

- For normal test plasmas, a 1/10 dilution can be used.
- For lower concentrations (for example, 0.75 g/l–1.5 g/l), the plasma should be diluted 1/5 (and the value read from the graph and multiplied by 5/10).
- For levels <0.75 g/l, the test plasma should be diluted 1/2 (and the value read from the graph and multiplied by 2/10).
- For higher levels (>4 g/l), the test plasma should be diluted 1/20 (and the value read from the graph and multiplied by 20/10).

The test is not affected by heparin at the levels used for the treatment of venous thromboembolism. The higher levels used for cardiopulmonary bypass can however prolong clotting times, leading to an underestimation of fibrinogen, unless the reagent contains heparin neutralizers to counter this.

**TYPICAL CALIBRATION DATA**

*Note: A calibration curve must be established with the reagents in local use.*

Standard plasma: 2.1 g/l fibrinogen

**Figure 18.1. Example of a fibrinogen calibration**

<table>
<thead>
<tr>
<th>Dilution of standard</th>
<th>Concentration of fibrinogen (g/l)</th>
<th>Clotting time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/5</td>
<td>4.2</td>
<td>8.5</td>
</tr>
<tr>
<td>1/10</td>
<td>2.1</td>
<td>14</td>
</tr>
<tr>
<td>1/15</td>
<td>1.4</td>
<td>19.5</td>
</tr>
<tr>
<td>1/20</td>
<td>1.05</td>
<td>24.5</td>
</tr>
</tbody>
</table>

Examples:

Test plasma 1: diluted 1 in 10, clotting time 15 seconds.
  fibrinogen = 1.9 g/l (from calibration graph)

Test plasma 2: diluted 1 in 5, clotting time 16 seconds.
  fibrinogen = 1.8 g/l from calibration graph × 5/10
  (since 1/5 dilution rather than 1/10)
  = 0.9 g/l
A number of coagulation analysers can estimate the level of fibrinogen during determination of prothrombin time. This is possible because the change in light scatter or transmission as a consequence of clot formation is proportional to the initial fibrinogen concentration. These methods are commonly referred to as PT-derived fibrinogen.

There are limitations to most of the PT-derived methods. In particular, the results obtained are often much higher than those obtained by Clauss assay when there are either very low levels (<1.5 g/l) or raised levels (above 5 g/l) of fibrinogen. Results are usually normal in the presence of dysfibrinogenemia. For a review of these issues, see Mackie et al. (2003).

There are Clauss fibrinogen methods that are suitable for assaying undiluted test plasma, but results may not be interchangeable with the results of the widely used Clauss assays employing diluted test plasma (Jennings et al. 2009).

REFERENCES
