Clotting test for monitoring anticoagulant therapy with hirudin

**Introduction**

Based on the interaction of hirudin with meizothrombin, simple ecarin clotting tests for the monitoring of anticoagulant therapy with hirudin have been developed and described in the literature (Nowak, 1996; Pötzsch, 1997). Whole blood of patients undergoing hirudin treatment is collected over heparin. A blood sample is provided with ecarin to convert prothrombin to meizothrombin which is inhibited by hirudin present in plasma. Unbound meizothrombin converts fibrinogen into fibrin whereby the clotting time is proportional to the hirudin concentration in the blood sample. A calibration graph plotting the hirudin concentration versus the ecarin clotting time was found linear over a range of 0.1 µg to 2.0 µg hirudin / ml. The ecarin clotting time proved also useful for the control of anticoagulant therapy with synthetic thrombin inhibitors, e.g. Argatroban or Efegatran. Furthermore Ecarin is used to determine prethrombin 1 and 2.

**Application**

Determination of prothrombin levels in patients undergoing anticoagulant therapy with hirudin or with synthetic inhibitors, e.g. Argatroban or Efegatran.

**Reagent**  
Code: 116-01

1 vial of Ecarin contains 50 Ecarin units.  
Store at +2 to +8°C. The lyophilized reagent is stable until the expiry date indicated on the box.

**Buffer**  
Code: 116-10

1 vial of HEPES buffer contains 12.5 ml.  
Store at room temperature. The ready for use buffer is stable until the expiry date indicated on the box.

**Sample**

Citrated plasma: Collect 9 vol. of venous blood into 1 vol. of 3.2 or 3.8 % trisodium citrate and mix gently. (In the USA: Follow NCCLS guidelines H3A2).  
Centrifuge for 10 minutes at 2500 g. The plasma can be stored for up to 8 hrs prior to analysis.

**Quality control**

Normal or pathological quality control plasmas should be run with each series.

**Preparation of the reagent**

Reconstitute the contents of 1 vial Ecarin with 1 vial of HEPES buffer. Concentration of Ecarin after reconstitution: 4 EU / ml.  
Store at +2 – +8°C. Avoid contamination.

**Stability after reconstitution**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>+37 °C</td>
<td>6 h</td>
</tr>
<tr>
<td>+2 – +8 °C</td>
<td>7 days</td>
</tr>
<tr>
<td>- 20 °C</td>
<td>6 months</td>
</tr>
</tbody>
</table>

**Pipetting scheme**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma/control</td>
<td>100 µl</td>
</tr>
<tr>
<td>Ecarin solution</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Determination of the clotting time at + 37°C

Ecarin concentration in the test: 1.33 EU / ml. 
Ecarin time test can be adapted on semi- or fully automated instruments. Follow the recommendations of the instrument manual.

**Expected values**

*Normal range:* The results are dependent on the technique used and on preanalytical factors. Each laboratory should determine its own normal range. Normal times of up to 37 secs. were found in a small number of samples in our laboratory with the manual assay. The clotting times may however vary from lab to lab, especially when semi- or automated coagulometers are used.

2 µg/ml Hirudin: Ecarin time of 74 secs was found.

**Other applications**

Due to its action on acarboxy prothrombin, ecarin can be used for the determination of acarboxy prothrombin in the supernatant of barium sulfate treated patient plasma where normal carboxylated prothrombin has been removed by adsorption. Ecarin may be used for the detection of low prothrombin concentrations in plasma fractions for quality- and in-process control purposes. Ecarin, used in conjunction with the phospholipid- and calcium ion-dependent prothrombin activator Textarin from *Pseudonaja textilis* venom, forms a highly sensitive and specific test system for lupus anticoagulant.

**Caution**

For research use only.

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