Pefakit® Reptilase®Time

Intended Use and Application

Pefakit® Reptilase®Time is a plasma based functional assay intended to be used for the determination of the Reptilase® induced clotting time (= Reptilase®Time, ReT) by a manual method or on a semi- or fully automated system. The ReT has been recommended for the investigation of the last phase of blood coagulation [1,2,3,4,5,6].

Introduction

Pefakit® Reptilase®Time reagent contains a standardized amount of Batroxobin, the thrombin-like proteolytic enzyme from Bothrops atrox venom. In contrast to thrombin which releases the fibrinopeptides A and B from fibrinogen, Batroxobin specifically splits off only fibrinopeptide A. Batroxobin does not inactivate or activate other hemostasis proteins or platelets.

In contrast to thrombin Batroxobin is not inhibited by heparin, heparinoids, antithrombin, hirudin, aprotinin or antifibrinolytic drugs. Fibrinogen degradation products (FDP), however, prolong the ReT by interfering with fibrin polymerization. The ReT can therefore be used for the detection of FDP and differentiating between fibrin synthesis disturbances and influence of heparin in combination with Thrombin Time (TT) [5].

Principle of the Method

A prolongation of the ReT can be expected in a variety of clinical conditions such as:

- fibrin polymerization disorders or inhibitors (e.g. myeloma proteins, autoantibodies against fibrinogen)
- afibrinogenemia or hypofibrinogenemia
- hyperfibrinolysis
- disseminated intravascular coagulation (DIC), e.g. in patients with septicemia or malignancies
- liver disease
- thrombolytic therapy

Due to its heparin insensitivity the ReT can detect fibrinogen polymerization disorders even in the presence of heparin. A normal ReT in a patient with prolonged thrombin clotting time can indicate the presence of heparin.

Reagent

The Reptilase®Time reagent contains 20 BU (batroxobin units) and stabilizers. One BU corresponds to the activity of approximately 0.17 NIH-unit of thrombin.

Material required but not provided

- Deionized water
- Calibrated pipettes (100 - 1000 μl)
- Centrifuge (2'500g) with timer
- Incubator (37 °C) with timer
- Glass or plastic tubes
- Normal and pathological quality control plasmas

Storage and Stability

The lyophilized reagent is stable and can be used up to the expiry date given on the label when stored unopened at 2 - 8° C.

Stability after reconstitution:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 °C</td>
<td>6 h</td>
</tr>
<tr>
<td>2 - 8 °C</td>
<td>5 days</td>
</tr>
<tr>
<td>- 20 °C</td>
<td>2 months</td>
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</tbody>
</table>

Quality Controls

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

Blood Collection and Sample Preparation

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 2'500 g. The plasma can be stored for up to 8 hrs prior analysis.

Preparation of the Reagent

Reconstitute the contents of one vial in 1 ml deionized water. Store the reconstituted solution at +2 to +8° C. Avoid contamination.

Procedure and Pipetting Scheme

Determination of the ReT by the manual method:

- Incubate the reagent at +37°C for at least 5 min. Prevent evaporation.
- Use clean plastic or glass tubes.
- Prewarm the tubes for at least 5 min. before use.
- Put 0.3 ml test or quality control plasma into the prewarmed test tube.
- Incubate the plasma at +37°C for 2 min.
- Add 0.1 ml Reagent to the plasma.
- Determine the clotting time in seconds (s).

The ReT test can be adapted on semi- or fully automated instruments. Follow the recommendations of the instrument manual. If possible use the sensitivity settings of the TT.
Expected Values

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 22 seconds 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.

Other Applications

Pefakit® Reptilase® Time (also known under its former name Reptilase®-Reagent) has been used for the determination of clottable fibrinogen or for the clot retraction test. Specific information is available on request.

Precautions

Contact with the proteolytic enzyme Batroxobin may cause allergic reactions. Avoid inhalation, skin and eye contact.

For in vitro diagnostic use only! Do not inject!

Bibliography


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