Prothrombin Time

Calcium thromboplastin for the determination of Prothrombin Time or one-stage Quick’s Time

SUMMARY
Coagulation may be produced by an extrinsic pathway (tissue damage) or by an intrinsic pathway (when blood contacts epithelium other than the normal vascular one).
The determination of Prothrombin Time or Quick’s Time is a global test to evaluate the extrinsic coagulation, being sensitive to: factor II or prothrombin, factor V or proaccelerin, factor VII or proconvertin and factor X or Stuart-Prower Factor.
Therefore, the test is used for:
- routine tests in pre-surgery analysis;
- detection of alterations in the levels of one or more factors involved in the extrinsic pathway;
- control of oral anticoagulants therapy.

PRINCIPLE
This assay is based upon measuring the coagulation time of a decalcified plasma, placed at 37°C and in the presence of an excess of tissue thromboplastin and calcium. This method does not detect deficiencies in the intrinsic pathway factors (VIII, IX, XI and XII).

PROVIDED REAGENTS
Reagent: vials containing rabbit brain thromboplastin, calcium chloride for a final concentration of 0.0125 mol/l, and sodium chloride for a final concentration of 0.1 mol/l.

NON-PROVIDED REAGENTS
Bidistilled or deionized water.

INSTRUCTIONS FOR USE
- Open a vial removing the metal precipit and slowly pulling out the rubber stopper to avoid any loss of material.
- Add the bidistilled or deionized water volume indicated on the label. Verify that the water temperature is not above 37°C.
- Cap and gently shake until a homogeneous suspension is obtained. Homogenize each time before use.

WARNING
The Reagent is for “in vitro” diagnostic use.

STABILITY AND STORAGE INSTRUCTIONS
Reagent: is stable in refrigerator (2-10°C) until the expiration date shown on the box.
Reconstituted Reagent: in refrigerator (2-10°C) is stable for 5 days from its reconstitution.

SAMPLE:
Plasma
a) Collection: obtain blood carefully (avoiding stasis and trauma) and place it in a tube with anticoagulant in an exact 9+1 proportion (e.g.: 4.5 ml blood + 0.5 ml anticoagulant). Mix gently. Centrifuge and separate the plasma before 30 minutes.
b) Additives: to obtain the plasma, 0.130 mol/l sodium citrate should be used.
c) Known interfering substances:
- contaminations, visible or not, yield falsely extended times;
- the presence of heparin or EDTA, void the results;
- visible hemolysis interfere with the photo-optical reading of the results.
See Young, D.S. in References for effect of drugs on the present method.

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REQUIRED MATERIAL (non-provided)
- Hemolysis tubes.
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at 37°C.
- Stopwatch.
- Light source for observing the clot.

PROCEDURE
1- Place plasma (unknown or control) in water bath at 37°C for 2-3 minutes (no more than 10 minutes).
2- In a hemolysis tube, place 0.2 ml reconstituted Reagent and preincubate at 37°C for 2-3 minutes (no more than 10 minutes).
3- Pipette 100 ul preincubated plasma and quickly add to the tube that contains the 0.2 ml Reagent, simultaneously starting the stopwatch.
4- Keep the tube in the water bath, near a light source. Before the estimated coagulation time, remove the tube from the bath, gently move back and forward one or two times per second and stop the stopwatch when the clot appears.
5- Calculate the average coagulation time of the determination, for each sample by duplicate (unknown or

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control). If the difference between the replicates of the same sample is higher than 5%, it is recommended to repeat the procedure discarding the previous values. If a measuring instrument is used, follow the manufacturer’s instructions.

INTERPRETATION OF THE RESULTS

The results can be expressed in different ways:

1- Prothrombin Time or Quick Time in seconds.

2- Prothrombin Activity Percentage in reference to a normal plasma (100% activity): a prothrombin activity curve from a pool of normal fresh plasmas should be done.

**Calibration curve**

In hemolysis tubes, prepare 5 dilutions (each one by duplicate) of a pool of at least 3 normal plasmas, according to:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1:1</th>
<th>1:2</th>
<th>1:3</th>
<th>1:4</th>
<th>1:8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity percentage (%)</td>
<td>100</td>
<td>50</td>
<td>33.3</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>Normal plasmas pool (ml)</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Saline solution (ml)</td>
<td>-</td>
<td>0.3</td>
<td>0.6</td>
<td>0.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Determine the Prothrombin time for each dilution, using the described PROCEDURE. On linear graph paper, draw the results in a coordinates system. Place the Prothrombin Times in seconds on the ordinate axis and the Prothrombin Activity Percentages on the abscissa axis. Each laboratory should draw its own calibration curve, corresponding to the reagent batch in use. Repeat with each new reagent lot.

3- International Normalized Ratio (INR)

Use the enclosed value chart to calculate it.

QUALITY CONTROL METHOD

Normal and pathological control plasma.

REFERENCE VALUES

The obtained values in normal patients range between:
- Prothrombin Time or Quick Time: 10-14 seconds.
- Prothrombin Activity Percentage: 70-100%

Each laboratory should set its own intervals or Reference values. For patients undergoing treatment with antivitamin K, a therapeutic range has been established that can be expressed as follows:
- Prothrombin Activity Percentage: 25 - 35%
- INR: 2.4 - 2.5

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE. Other causes of erroneous results are:
- Erroneous collection of venous blood.
- Variations in the ratio anticoagulant/sample or in the citrate concentration used affect the Quick Times, thus, it is recommended to control the anticoagulant dose used when collecting the sample.
- Preincubation in the 2nd step of the PROCEDURE should not exceed 10 minutes, indicated as maximum limit. On the other hand, it is recommended to remove the re-constituted reagent from the refrigerator just before performing the test and to put it back after finishing. The repeated exposure of the reagent to room temperature for several hours deteriorates it, causing an extension of the prothrombin times.

PERFORMANCE

a) Reproducibility: processing replicates of the same samples on the same day, the following results were obtained:

<table>
<thead>
<tr>
<th>X (n = 20)</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5 sec</td>
<td>± 0.4 sec</td>
<td>3.5 %</td>
</tr>
<tr>
<td>21.2 sec</td>
<td>± 0.6 sec</td>
<td>2.8 %</td>
</tr>
</tbody>
</table>

b) Comparison with a reference method: processing different samples with Prothrombin Time and with other method taken as reference, it was observed:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>X (n = 60)</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin Time</td>
<td>13.2 sec</td>
<td>± 0.3 sec</td>
<td>2.3 %</td>
</tr>
<tr>
<td>Reference</td>
<td>12.8 sec</td>
<td>± 0.3 sec</td>
<td>2.3 %</td>
</tr>
</tbody>
</table>

KIT SIZE

Kit for 100 tests (10 x 2 ml) (Cat-Nr.: 411).

REFERENCES