QUANTITATIVE MEASUREMENT OF FACTOR V-LEIDEN WITH A NEW, ONE STEP, CALIBRATED, CLOTTING ASSAY

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Introduction

- We developed a new clotting method for quantitatively measuring FV-L (Factor V Leiden) concentration in citrated plasma, by its resistance to the action of Activated Protein C (APC).
- Normal FV is not measured in that assay, as it is totally inhibited by APC, whilst FV-L keeps its clotting activity.

Methods

1. Principle

The diluted plasma is mixed with a purified clotting factor mixture, in a constant and optimized concentration, (R1 : Fibrinogen, Prothrombin, Protein S and APC). Purified FXa, with phospholipids (R2), is then added. Coagulation is initiated by the addition of calcium (Ca++) and the clotting time (CT) is recorded. The CT obtained is inversely proportional to the FV-L concentration. An inverse linear relationship is obtained, on lin-log coordinates, between the CT and the FV-L concentration.

2. Assay calibration

Calibration is performed using various mixtures of a (R506Q) heterozygous plasma pool (for which the FV-L concentration corresponds to 50 % of that of total FV), and a normal plasma pool (containing by definition 0 % FV-L and 100 % of normal FV).

The standard assay dilution being 1:20, the 1:20 heterozygous plasma pool dilution contains 50% Factor V-L and the 1:10 dilution, 100%. The 1:1 mixtures of the heterozygous and the normal plasma pool mixture, diluted 1:20, corresponds to 25 % FV-L, and the mixture of one part of the FV-L heterozygous pool with 4 parts of the normal pool, diluted 1:20, corresponds to 10 % FV-L.

<table>
<thead>
<tr>
<th>FV-L (%)</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pool</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Heteroz. pool</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dil.</td>
<td>1:10</td>
<td>1:20</td>
<td>1:20/1:20</td>
<td></td>
</tr>
</tbody>
</table>

The 1:20 diluted normal pool contains no Factor V-L.

Clotting times measured range from about 30 seconds for 100 % FV-L to 100 seconds for normal plasmas.

3. Protocol

- Clotting mixture containing human Fibrinogen, Activated Prothrombin, Protein S supplemented with human Activated Protein C and a heparin neutralizing substance, lyophilized.
- Purified Human Factor Xa containing rabbit brain phospholipids, lyophilized.
- CaCl2 0.025M, Reagent not supplied.

Coagulation is initiated by the addition of calcium (Ca2+) and the clotting time (CT) is recorded.

Results

Factor V-Leaden concentrations in normals and patients carrying the (R506Q) mutation:

<table>
<thead>
<tr>
<th>Patients</th>
<th>FVL Conc. (%)</th>
<th>CT (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI (N=30)</td>
<td>&lt;10</td>
<td>&gt;100</td>
</tr>
<tr>
<td>HTZ (N=8)</td>
<td>35 - 60</td>
<td>50-70</td>
</tr>
<tr>
<td>HMZ (N=2)</td>
<td>&gt;100</td>
<td>&lt;40</td>
</tr>
</tbody>
</table>

* CT may vary from lot to lot, but FVL-L concentrations are accurately determined by using the calibration curve specific for each lot and each run.

Conclusions

- A totally quantitative assay for the measurement of FV-L concentrations on citrated plasma is presented.
- Only one clotting test is required (= no problem of result interpretation).
- Excellent discrimination (for both clotting times and FV-L concentrations) between normals, heterozygous and homozygous patients (for the R506Q mutation).
- Patients with low concentrations of total FV clotting activity (<25%) must be identified, and the diagnosis confirmed by comparing FV-L and total FV clotting activity (normals < 0.1).
- No interference of Heparin or Dicoumarol therapy.
- Easy to perform, cost effective and reliable assay, fully automatable on laboratory instruments.

References

1. Dahiback B. et al. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C : prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 1993; 90(3); 1004-8.