CLASSIFICATION OF FACTOR V-LEIDEN CARRIERS BY QUANTITATIVELY MEASURING ITS PROCOAGULANT ACTIVITY COMPARATIVELY TO THAT OF FACTOR V

HEMOCLOT Quanti. V-L is now CE Marked and 510(k) approved

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Introduction

- Presence of FV-L (Factor V Leiden: R506Q mutation) is usually evidenced with clotting methods using the clotting time ratio of a two step assay performed with or without activated Protein C (APC).
- Genetic status of FV-L carriers is confirmed with molecular biology. When the APC-r ratio is used, there is sometimes overlapping between heterozygous and normal plasmas and the assay is only qualitative.
- We used a new quantitative clotting assay (HEMOCLOT Quanti-V-L – ACK065K) for measuring FV-L in plasma, in normals and patients.
- The aim of this study was to test citrated plasma from normal, heterozygous and homozygous patients for FV-L, using this new method comparatively to the conventional assay performed in the absence, or presence, of APC.

Methods

1. Principle and reagents

HEMOCLOT Quanti V-L (ACK065K): 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 (Ca2+) and the clotting time (CT) is measured. The CT obtained is inversely proportional to the FV-L concentration. An inverse linear relationship is obtained, on log-log coordinates, between the CT and the FV-L concentration.

- Calibration between 0 and 100 % of FV-L, using 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).

HEMOCLOT Factor V-L (ACK061K): Clotting assay performed without or with APC and calculating the CT ratio (APC-r ratio).

Both assays are performed using automatic methods on STA-R.

2. Blood collection

Blood was collected on 0.109M or 0.129M citrate anticoagulant centrifuged at 3,000g for 20 min at 18°C or below and plasma decanted into a plastic tube.

Tested samples: Normal plasmas (Nl, N=30) (from a French blood bank), plasmas of patients carrying the R506Q mutation (FVL) identified as heterozygous (HTZ, N=61) (including 19 Dicoumarol treated) and homozygous (HMZ, N=18) (all from H. Mondor Hospital, Créteil, France).

Molecular biology was used for classifying patients as heterozygous or homozygous and performed at H. Mondor Hospital.

Results

- FV-L was quantitated in the various groups and allowed discriminating accurately between patients without or with FV-L.
- Normal plasma containing only normal FV has always: FV-L <10%.
- In this study, plasmas from patients with FV-Leiden identified as:
  - Heterozygous contained >25% and <75% FV-L (no interference of Dicoumarol therapy).
  - Homozygous contained >70% FV-L.
- The FV-L/FV clotting activity ratio duly confirmed the classification established and complies with the genetic status.
- This assay offers a single and easy way to diagnose patients carrying FV-L.
- It is recommended to measure FV clotting activity, when a FV decreased concentration is suspected (<25%).

Conclusions

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