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QUANTITATIVE MEASUREMENT OF FACTOR V LEIDEN IN HETEROZYGOUS AND HOMOZYGOUS PATIENTS FOR THE R506Q FACTOR V MUTATION

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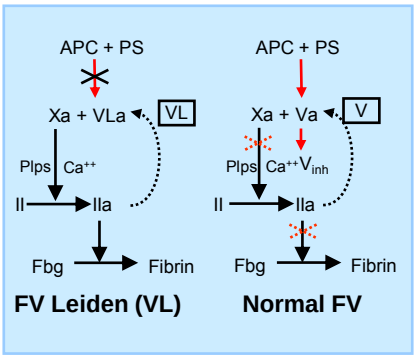
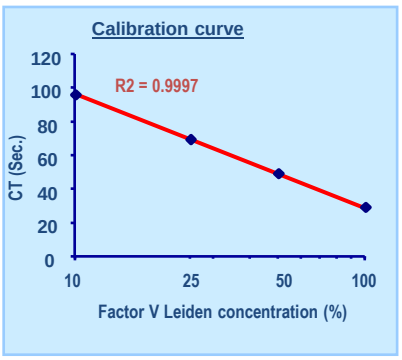
INTRODUCTION

- Presence of FV-L (Factor V Leiden: R506Q mutation) is usually evidenced with clotting methods using the clotting time ratio, using 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, from normals and patients with APC-Resistance (confirmed by Molecular Biology for R506Q mutation).
- 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.



ASSAY CALIBRATION FOR HEMOCLOT QUANTI V-L

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).



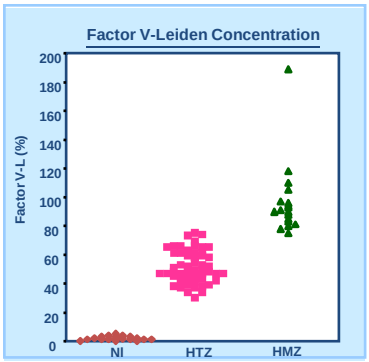
- ✓ The standard assay dilution being **1:20**, the **1:20** heterozygous plasma pool dilution contains **50%** Factor V-L, and the **1:10** dilution, **100%**.
- ✓ The **1:1** mixtures of the heterozygous and the normal plasma pools mixture, diluted **1:20**, corresponds to **25 % FV-L.**
- ✓ The mixture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

Results obtained with qualitative and quantitative methods on Normal and Abnormal controls.

	ACK061K (ratio)		ACK065K (%)	
	Exp. Values	FV-L ratio	Exp.values	% FV-L (STAR)
Normal control	2.56	2.15	<5%	1
Act PCR Abnormal control	1.70	1.69	51 [41-61]	46

HEMOCLOT Quanti. V-L, Normal Control and Act PC-r Control are now CE Marked and 510(k) approved

- 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 plasmas contained between **>25% and <75% FV-L** (no interference of Dicoumarol therapy).
  - Homozygous plasmas contained >70% FV-L.



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 lin-log coordinates, between the CT and the FV-L concentration.
- **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.
- FV clotting activity was measured with **Hemoclot Factor V Reagent** (ACK071K) and Factor V antigen with **Zymutest Factor V** (ARK009A).
- FV assays were calibrated using the **NIBSC** secondary standard, lot 2.

2. BLOOD COLLECTION

- Blood was collected on 0.109M or 0.129M citrate anticoagulant centrifuged at 3,000g for 20 mn at 18°C or below and plasma decanted into a plastic tube.
- Tested samples: Normal plasmas (NI, 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 obtained for each group of patients with both FV-L methods

Patients		Ratio	Quanti V-L (%)
NI (N=30)	Mean Min-Max	2.22 2.05-2.44	<10 <10
HTZ (N=61)	Mean Min-Max	1.72 1.56-1.84	50.2 27-75
HMZ (N=18)	Mean Min-Max	1.43 1.24-1.49	90 73-188

➢ Excellent classification of normal, heterozygous and homozygous with the quantitative method

Determination of the Factor V clotting activity and Factor V antigen for each group of patients

Patients	FV-L (%)	FV:Ag (%)	FV clotting (%)	FVL/FV ratio (%)
Normal	<10	93	107	<0.05
HTZ (N=42)	49	102	89	0.55
HTZ* (N=19) *Dicoum. treated	52	108	85	0.62
HMZ	90	106	71	1.30

➢ Calculating FV-L/FV:Ag or FV-L/FV:clotting ratios show:
- Normals < 0.1
- Heterozygous : 0.5 ± 0.1
- Homozygous : 1.00 ± 0.2

CONCLUSIONS

- This new clotting method allows an accurate and quantitative measurement of Factor V Leiden clotting activity (resistant to the action of Activated Protein C) and could be very useful for routine classification of patients carrying the Factor V Leiden mutation. But only molecular biology allows confirming the diagnosis, and classifying paitents as heterozygous or homozygous.
- Only one clotting test is necessary, and result is fully quantitative.
- This assay offers a single and easy way to diagnose patients carrying FV-L.
- It is recommended to also measure FV clotting activity, when a FV decreased concentration is suspected (<25%), and to calculate the FV-L/FV clotting ratio.
- The **FV-L/FV** clotting activity ratio duly confirmed the classification established and complies with the genetic status.
- Quantitation of Factor V Leiden could be an helpful tool for grading the thrombotic risk in patients with the R506Q Factor V mutation.

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