

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

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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 lin-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.

FV clotting activity was measured with **Hemoclott Factor V Reagent (ACK071K)** and Factor V antigen with **Zymustest Factor V (ARK009A)**.

FV assays were calibrated using the **NIBSC** secondary standard.

**Table 1:** CT obtained with the calibrator. The 1:20 dilution for each calibrator corresponds to 100, 50, 25 and 10%.

Calibration	% FVL	CT (sec)
Internal reference lot 63402 (CT in sec)	100	27.4
	50	41.5
	25	53.0
	10	69.0
	R <sup>2</sup>	0.999

**Table 2:** 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)
NI control	2.56	2.15	<5%	1
Act PCR Abnl control	1.70	1.69	51 [41-61]	46

## References

- Bertina RM and al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369(6475): 64-7.
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- Crookston K.P. and al. False negative factor V Leiden assay following allogeneic stem cell transplant. Br J Haematol 1998; 100: 600-2.
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## Conclusions

- 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%).

### 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

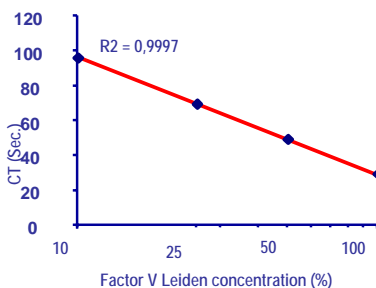
Results obtained for each group of patients with both FV-L methods

Patients		Ratio	Quanti V-L %
NI (N=30)	Mean	2.22	<10
	Min-Max	2.05-2.44	<10
HTZ (N=61)	Mean	1.72	50.2
	Min-Max	1.56-1.84	27-60
HMZ (N=18)	Mean	1.43	88
	Min-Max	1.24-1.49	73-118

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 (%)
NI	<10	93	107	<0.05
HTZ (N=42)	49	102	89	0.55
HTZ* (N=19) <small>*Dicoum. treated</small>	52	108	85	0.62
HMZ	90	106	71	1.30

### Calibration curve



### Factor V-Leiden Concentration

