

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 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 ²	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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- Labrousse S. and al. Molecular mechanism for APC resistance in the absence of Arg 506 mutation: factor V gene sequencing strategy. Thromb Res 1997; 87(2): 263-7.
- Crookston K.P. and al. False negative factor V Leiden assay following allogeneic stem cell transplant. Br J Haematol 1998; 100: 600-2.
- Castoldi and al. Expression of the normal Factor V allele modulates the APC resistance phenotype in heterozygous carriers of the Factor V Leiden mutation. J Thromb Haemost 2005; 3(12): 2695.

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

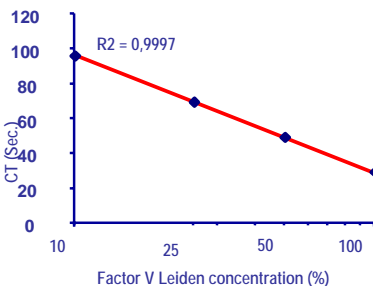
Results obtained for each group of patients with both FV-L methods

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

