

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

Amiral J, Peyrafitte M, Vissac AM  
HYPHEN BioMed Research, 95000 Neuville sur Oise (France)

## 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<sup>2+</sup>) 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).

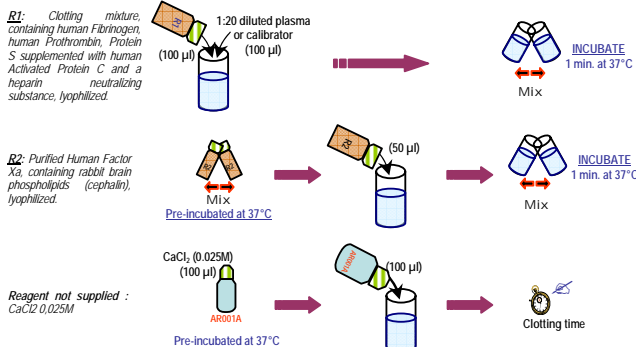
The standard assay dilution being 1:20, the 1:20 heterozygous plasma pool dilution contains 50% Factor V-L, and the 1:10 dilution, 100%. The 1:1 mixtures of the heterozygous and the normal plasma pools mixture, diluted 1:20, corresponds to 25 % FV-L, and the mixture of one part of the FV-L heterozygous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

FVL (%)	100	50	25	10
Normal pool	0	0	1	4
Heteroz. pool	1	1	1	1
Dil.	1:10	1:20	1:20	1:20

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



## 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.

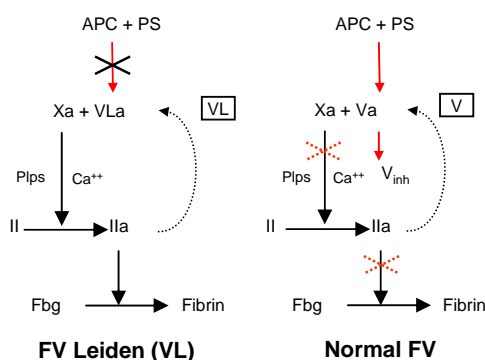
## Results

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

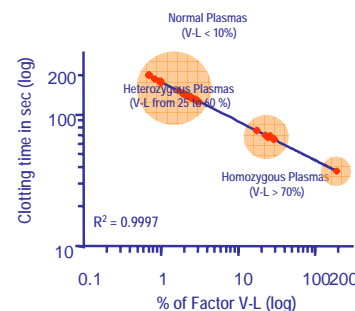
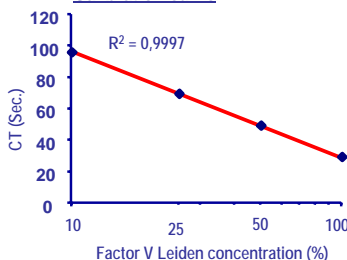
Patients	FVL Conc. (%)	CT* (sec)
NI (N=30)	<10	>100
HTZ (N=8)	35 - 60	50-70
HMZ (N=2)	>100	<40

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

## Scheme of the assay principle



### Calibration curve



## References

- Dahlback 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.
- Brenner B. et al. Activated protein C resistance can be associated with recurrent foetal loss. Br J Haematol 1997; 97: 551-4.
- Rosendaal F.R. et al. Factor V Leiden (Resistance to Activated Protein C) increases the risk of myocardial infarction in young women. Blood 1997; 89 (8): 2817-21.
- Chao-Hung Ho. Prevalence of Activated Protein C Resistance in the Chinese Population. Thromb Res 1997; 88: 409-12.