

## A NOVEL, SPECIFIC AND QUANTITATIVE ASSAY FOR MEASURING FACTOR V-LEIDEN, WITH A SINGLE STEP CLOTTING METHOD.

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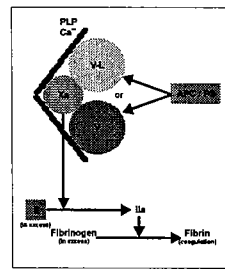
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### INTRODUCTION

Presence of Factor V-Leiden is measured with clotting methods, usually APTT, performed with or without Activated Protein C (APC). If this mutated Factor V (RQ 506) is present, there is a lack of prolongation of clotting time when APC is present and there is a reduced ratio of the clotting times performed with or without APC. These assays are highly dependent on reagents used and are difficult to standardise.

We present a new method which allows measuring quantitatively Factor V-Leiden, using a one step clotting assay, which is calibrated with variable mixtures of heterozygous plasmas for Factor V-Leiden and normal plasma, and which is performed in presence of APC. Clotting is triggered by a mixture of Factor Xa, phospholipids and calcium.

### PRINCIPLE



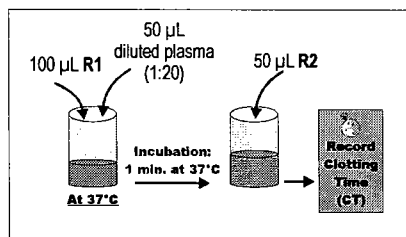
### ASSAY CALIBRATION

Calibration is made with a pool of heterozygous plasmas, from patients carrying the RQ 506 mutation, undiluted (the Factor V-Leiden concentration is then of 50%) or diluted with normal plasma: 1:2 (25% FV-L), 1:4 (12.5% FV-L) and 1:8 (6.25% FV-L); Normal plasma has a 0% Factor V-Leiden concentration. These calibration plasmas are then used diluted 1:20 for the assay (therefore the heterozygous plasma pool for Factor V-Leiden, diluted 1:10 is the 100% Factor V-Leiden concentration).

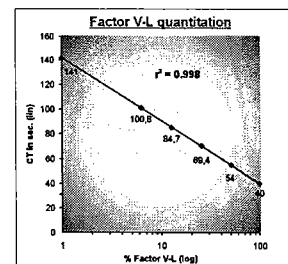
### MATERIALS AND METHODS

- Owren Koller or Imidazole dilution buffer
- Factor Xa
- Activated Protein C, human
- Phospholipids from rabbit brain
- Calcium
- Clotting mixture containing Fibrinogen, Prothrombin, Protein S and APC (R1)
- Reagents used:
  - R1: Clotting mixture
  - R2: Factor Xa with phospholipids and calcium

### PROTOCOL



### CALIBRATION CURVE

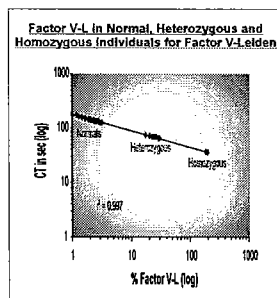


There is an inverse relationship between concentration of Factor V-L and clotting time.

### RESULTS

- Normals: < 5% V-L
- Heterozygous: 25-60% V-L
- Homozygous: > 75% V-L

(If necessary measure FV)



### CONCLUSIONS

- ▶ We present a new **Quantitative** reagent for measuring **Factor V-Leiden**.
- ▶ There is **no interference** of plasma factor deficiencies (other than that of Factor V, the factor measured). (Measurement of Factor V and its comparison with the level of Factor V-L can then be useful in some patients with low concentrations of this factor).
- ▶ **Excellent discrimination** between heterozygous, homogygous and normals.
- ▶ This **one step method** is performed using only a **clotting time (CT)**.

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