

Evaluation of a Novel Quantitative Assay for Factor V Leiden

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Introduction

- Hemoclot Quanti F-L (Quadrach, UK) is a clotting assay designed to measure Factor V Leiden (FVL) levels in citrate plasma.
- The assay is performed in the presence of activated protein C (APC) and protein S (PS) with the prolongation of the FXa triggered clotting time being inversely proportional to the concentration of FVL (%FVL).

Aim

- Evaluate Quanti F-L assay ability to discriminate between FVL subtypes; heterozygous (Het), homozygous (Hom) and wildtype (Wt).
- Compare Quanti F-V to Coatest APC Resistance V assay (Chromogenix).

Subject Groups

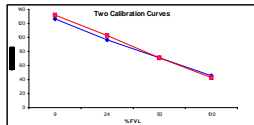
- 39 FVL wildtypes
- 33 Heterozygous FVL
- 3 Homozygous FVL
- Wildtype status established utilising PCAV screening test (Diagnostic Reagents, UK)
- Het and Hom status evaluated using LightCycler (Roche Diagnostics)

Method

- The assay is performed on dilute test plasma to which a reagent containing fixed concentrations of prothrombin, fibrinogen, APC and PS is added.
- A second reagent containing purified FXa and phospholipid is added with clotting initiated through the addition of CaCl_2 (0.025M).

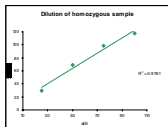
Calibration

- All testing performed on an ACL Top (IL, UK).
- Three human plasmas (Hyphen, BioMed) reconstituted and diluted in buffer are equal to 9, 24, 50 and 100% FVL.
- Two calibration curves performed generated R2 values of 0.994 and 0.999.



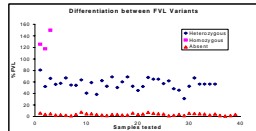
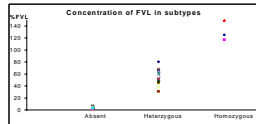
Precision

- A FVL absent control material tested (n=10) generated coefficient of variation (CV) of 11.2%.
- A known heterozygous (n=6) generated CV of 3.6%.
- A known FVL hom was diluted to assess how well the assay estimates FVL at different concentrations.



Results

Group	Quanti F-L Mean (Range)
Wt (n=39)	3.6 (0.8-7.5)
Het (n= 33)	57 (31-81)
Hom (n= 3)	131 (117-150)



- There was no significant difference between %FVL in warfarinised (n=12, mean 57.4%) and non warfarinised patients (n=21, mean 58.8%) in heterozygotes (p=0.6623).

Coatest APC-R-V

- Coatest APC-R is a commonly used screening tests for FVL.
- Patient samples diluted in FV deficient plasma have APTT performed in the presence and absence of APC.
- Ratio of clotting times calculated.
- A subset of patients were tested by both methods and results compared.

Group	Quanti F-L Mean (Range)	Coatest Mean (Range)
Wt (n=29)	3.9 (0.8-7.5)	3.1 (2.5-3.8)
Het (n= 13)	56 (31-68)	1.9 (1.6-2.1)
Hom (n=2)	137 (125-150)	1.2 (1.2-1.3)

Conclusion

- Hemoclot Quanti F-L is a simple assay to perform on ACL Top and demonstrates strong differentiation between patient groups in samples tested.
- Assay compares well with the Coatest APC Resistance assay.
- Quantification of %FVL may be useful but requires further studies