Feasibility of a new automated FXIII activity assay

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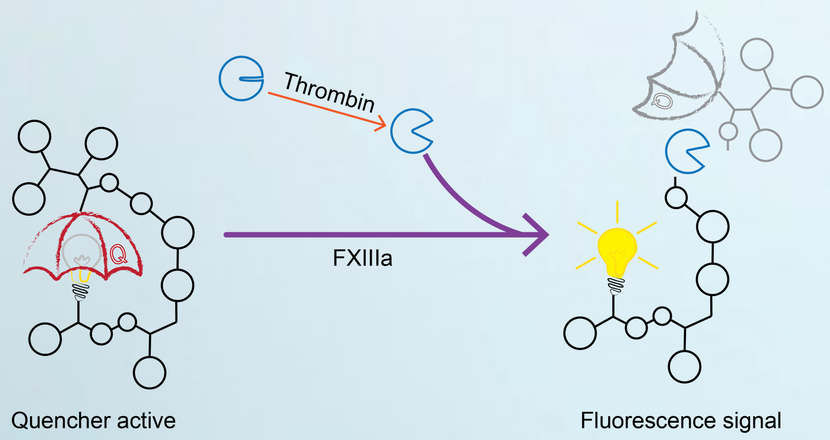
INTRODUCTION

FXIII plays a pivotal role in the terminal phase of blood coagulation by cross-liking the fibrin network and therefore stabilizing the formation of mature blood clots. Deficiency of FXIII occurs rarely but activity levels found ≤ 30% may be associated with severe bleeding. Even if there are several manual commercially available FXIII assays, a robust automated activity assay is needed.
The aim of this study was to develop a fully automated assay using a fluorescence quencher method for the determination of FXIII activity. For this purpose a new coagulation analyzer, equipped with an optical Quenching module was co-developed.

MATERIALS AND METHODS

Assay principle

The newly developed TECHNOFLUOR FXIII Activity is based on the use of a highly sensitive fluorogenic substrate in combination with a thrombin reagent: Thrombin activated FXIIIa cleaves a dark quenching molecule from the side chain of a peptide incorporating glycine methyl ester. Subsequently, the fluorescence of an N-terminal coupled dye increases and can be monitored.



A newly developed automated analyzer with Quenching module (Ceveron s100), combining routine and speciality haemostasis testing, was used.

RESULTS

FXIII specificity

To provide evidence that this testsystem is specific for FXIII, plasma samples were measured with and without the FXIII specific inhibitor 1,3,4,5-Tetramethyl-2-[(2-oxopropyl)thio]imidazolium chloride (T101). Presence of the inhibitor abolished all measurable FXIII activity. Additionally, FXIII immunodepleted plasma also did not show any change in fluorescent signal.

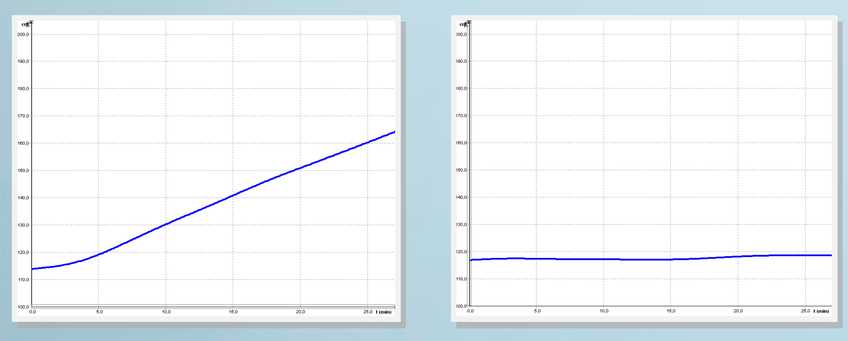


Fig 1. FXIII measurement raw data curve without (left) and with inhibitor (right).

Calibration

With an assay time of <30 min a calibration curve ranging from 0 - 0.8 IU/mL FXIII activity could be established using SSCLOT4.

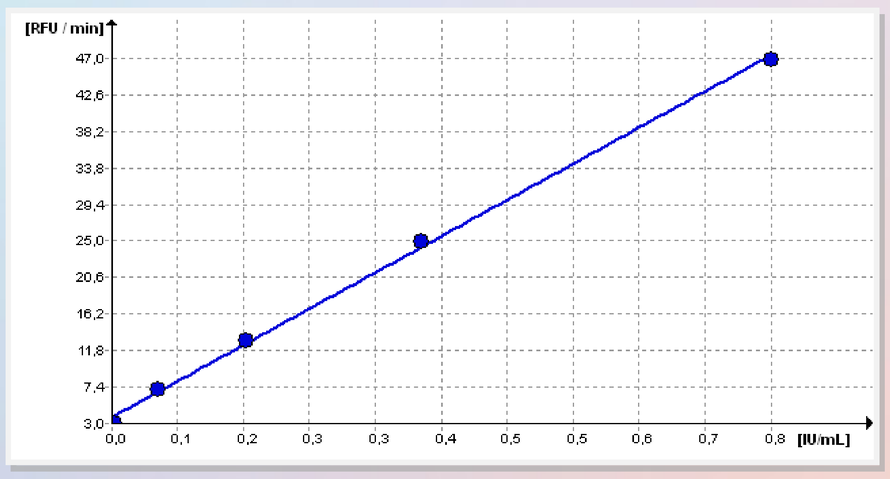


Fig 2. typical calibration curve on Ceveron s100; R^2 = 0.9985

Stability

To investigate the stability of the new FXIII activity test four samples with different activity levels were tested fresh reconstituted and after 24h storage.

	FXIII Activity	Recovery
Sample 1	0.7 IU/mL	98.3 %
Sample 2	0.4 IU/mL	97.8 %
Sample 3	0.2 IU/mL	97.4 %
Sample 4	0.04 IU/mL	99.9 %

Accuracy

To study the correctness of the newly developed assay, EQA samples from ECAT were analyzed and compared to the survey results. In addition, the new SSCLOT5 was measured.

Sample	Assigned value	Measured value
17.114	0.04 IU/mL	0.02 IU/mL
17.115	0.11 IU/mL	0.11 IU/mL
18.156	0.17 IU/mL	0.15 IU/mL
SSCLOT5	0.77 IU/mL	0.76 IU/mL

CONCLUSIONS

The fully automated TECHNOFLUOR FXIII Activity assay run on the new Ceveron s100 haemostasis analyzer is an appropriate method for fast, accurate determination of this critical factor in the stabilization of blood clots. With a high degree of linearity over a wide assay range, this new assay shows excellent recovery of EQA samples, demonstrating high agreement with other FXIII activity assays on the market.

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