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Assessment of Factor IX Padua activity with a one-stage clotting method and with FXIa- and tissue factor/FVIIa-based chromogenic methods.

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INTRODUCTION

Factor IX (FIX) Padua, characterized by the point mutation Arg338Leu, is currently explored both in recombinant and gene therapy projects. FIX Padua shows up to 8-fold higher activity vs wild-type (wt) FIX in one-stage (OS) methods. Limited data are available with chromogenic substrate (CS) methods.
Aim: Determination of FIX Padua activity with two CS methods and one OS method.

MATERIALS AND METHODS

Analyses were made on the FIX Padua proband, his affected brother and unaffected father and aunt.
Methods: 1) OS method with Actin FS on BCS XP (Siemens, Germany) 2) FXIa-based CS Rox Factor IX kit (Rossix, Sweden) in microplates 3) In-house microplate TF/FVIIa-based CS method using Recombiplastin 2G (Instrumentation Laboratory, USA), Phospholipid-TGT (Rossix) and hFVIIa (Hematological Inc., USA) were used at dilution 1:170, 15 mM and 0.4 nM, respectively (final assay conditions). Sample concentration during activation was 0.67 vol% and the FIX activation time was 15 min at 37C. Generated FIXa was determined with the Rox FIX-A kit (Rossix). FIX antigen was determined with Asserachrom ELISA kit (Diagnostica Stago, France). Human FXIa (Enzyme Research, USA) was used for tests with increased FXIa levels in the Rox Factor IX method. Freshly frozen normal plasma (NPL, Precision Biologics, Canada) was used for comparison with FIX Padua.
Calibrator: SSC/ISTH Plasma, lot#4.

RESULTS

Fig 1 shows that the FIX activities for FIX Padua individuals were about two-fold higher with the OS method vs Rox Factor IX and the TF/FVIIa method, which both were in fair agreement.

Table 1 shows the specific activities (activity /antigen) derived from the data in Fig 1. For the Proband and his affected brother the specific activities were more than two-fold higher with the OS method in comparison with the two chromogenic methods. In contrast, the specific activity was close to 1 for unaffected family members with all methods.

Table 2 shows that no difference was obtained for the FIX Padua / NPL activity ratio when increasing the FXIa concentration up to 11-fold in the Rox Factor IX kit method.

Activation kinetics studies in the Rox Factor IX method showed a more than 2-fold faster FXa generation for FIX Padua vs wtFIX throughout the investigated time range 1-8 min and with no difference of relative rates vs time, supporting that the reported data with Rox Factor IX, using 8 min activation time, are representative for this chromogenic concept (data not shown).

CONCLUSIONS

- The specific activity (FIX:C/FIX Ag) of FIX Padua with Rox Factor IX was >3-fold higher than for wtFIX but only about 40% vs a OS method (FIX:C/FIX:Ag about 8.5).
- A TF/FVIIa based method showed a specific activity for FIX Padua of about 3.9, in fair agreement with Rox Factor IX.
- The ratio of FIX Padua activity vs wtFIX was not affected over an 11-fold increased FXIa concentration in the Rox Factor IX method.
- Both chromogenic and one-stage methods can be used to determine FIX Padua activity. Further studies are required to elucidate causes of method discrepancies.

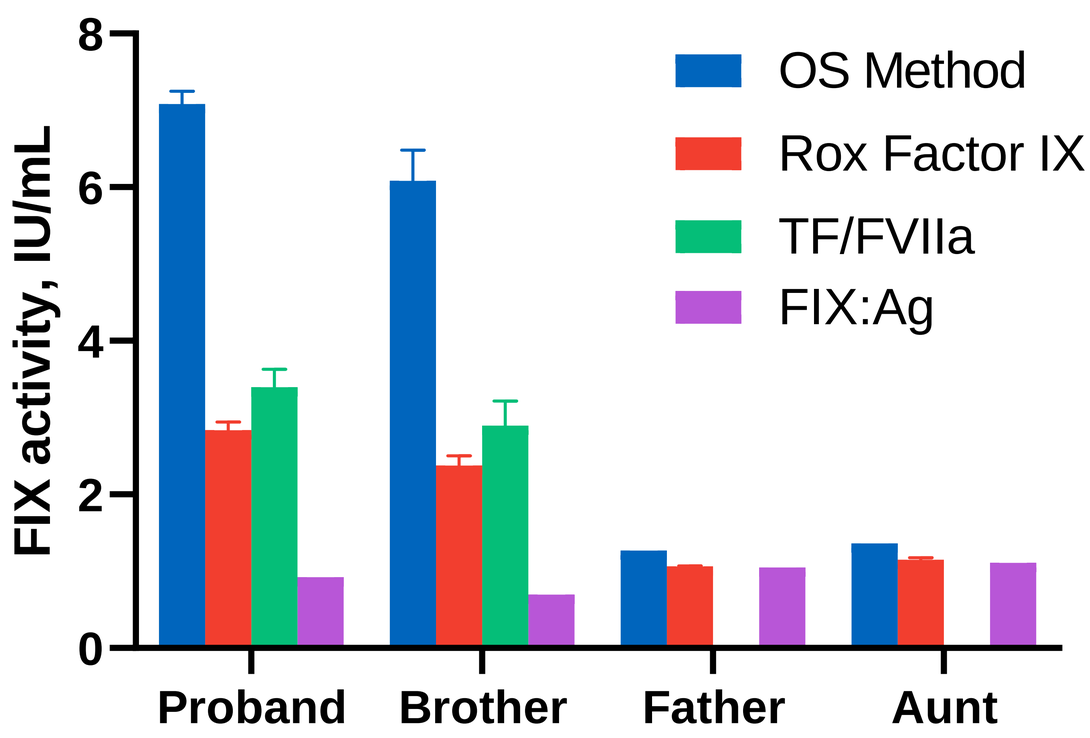

FIGURE 1 Assigned FIX activity (IU/mL) with different methods compared with FIX antigen.

TABLE 1 Specific activity (FIX:Act / FIX:Ag) obtained with different methods.

	OS method	Rox Factor IX	TF/FVIIa
Proband	7.7	3.1	3.7
Brother	8.7	3.4	4.1

TABLE 2 FIX activity ratio at increasing FXIa activity in Rox Factor IX.

	x1	x1.5	x 3.5	x11
Ratio FIX Padua / NPL	2.4 ± 0.4	2.6 ± 0.5	2.8 ± 0.2	2.6 ± 0.1

The effect of increasing FXIa activity in the Rox Factor IX kit method was determined and expressed as the FIX activity ratio of the affected brother and normal plasma (NPL). The range covers the FXIa activity generated in OS methods.

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