PB0099

Evaluation of Lupus Anticoagulant interference in a chromogenic Factor IX kit for determination of Factor IX activity.

P Bryngelhed[1], R Hengeveld[2], C Klopper-Tol[2], F Ehklas[2], S Rosén[3], AK Stroobants[2]

1Rossix AB, Mölndal, Sweden; 2Amsterdam UMC, University of Amsterdam, Dept. of Clinical Chemistry, Amsterdam, the Netherlands; 3Private Consultant, Kallered, Sweden

Introduction

Presence of Lupus Antibodies (LA) may interfere in one-stage (OS) Factor IX (FIX) methods, resulting in underestimation of FIX activity. Information is lacking regarding interference in chromogenic substrate (CS) methods.

Aim

Evaluation of LA interference in a chromogenic FIX kit

Method

dRVVT and Silica Clotting time methods were used to collect samples with LA positivity in at least one method. Positivity defined as ratio > 1.2. FIX activity was determined using Rox Factor IX (Rossix AB, Mölndal, Sweden) on ACL TOP 700 (IL, USA), STA-R Evolution (Stago, France) and a manual microplate method. Results were compared to a LA insensitive OS method with Actin FS (Siemens, Germany) performed on CS-2500 (Sysmex, Japan). Five samples with strong LA were reanalysed after 4-fold dilution with FIX deficient plasma (Stago). Strong LA was defined as dRVVT ratio ≥ 1.7; SCT ratio ≥ 2.6. Supplementary analyses were made on five LA positive plasmas for which a LA sensitive OS method with PTT LA (Stago) also was used.
Sample 1-5: SCT-positive, 6-12: dRVVT-positive, 13-17: SCT- and dRVVT-positive, 18-25: Strong LA

Results

Bias plots on % relative deviation of results with Rox Factor IX versus the LA insensitive OS method on analysis of 25 plasmas showed no trend towards any deviation at increasing LA strength (Figure 1).

Table 1 shows the results for five samples with strong LA activity after 4-fold predilution with FIX deficient plasma. Similar results were obtained in all cases between the original result and after 4-fold predilution, supporting lack of LA interference with Rox Factor IX.

Further more, table 2 shows a clear inhibition in the LA sensitive method PTT-LA with higher FIX potency at increasing dilutions, whereas Rox Factor IX and Actin FS gave similar results between dilutions.

Conclusion

- There is no interference of LA in the determination of FIX activity with the chromogenic Rox Factor IX kit as shown on analysis of 25 LA positive samples of which 8 were strongly positive.
- The Rox Factor kit can be safely used for determination of FIX activity also in plasma from LA positive patients.

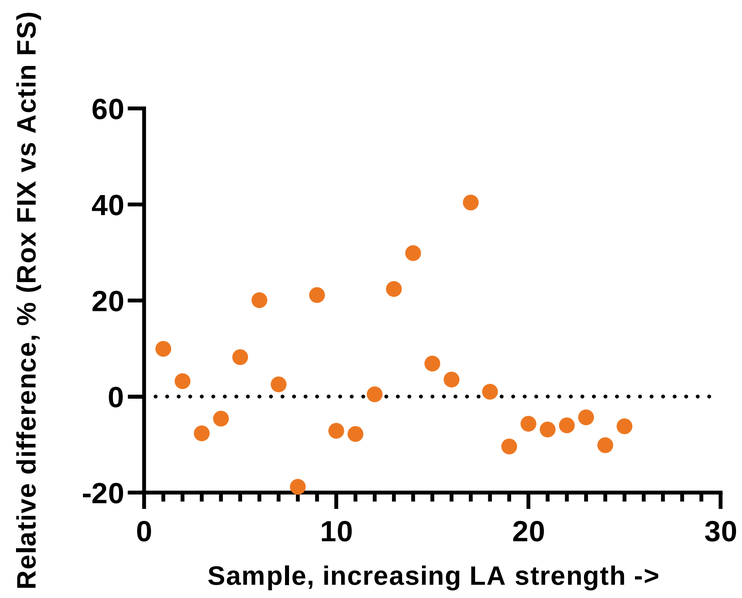

Fig. 1: Relative difference in Factor IX potencies (Rox Factor IX vs Actin FS) for 25 LA positive samples. Rox Factor IX sample dilution 1:80 and Actin FS 1:20.

	Actin FS			Rox Factor IX		
Sample no	Dil 1:20	Pre dil x4 in FIX def plasma	% vs 1:20	Dil 1:80	Pre dil x4 in FIX def plasma	% vs 1:80
18	148	150	101	150	141	94
20	68	74	110	64	60	94
21	136	153	112	127	125	98
23	90	95	105	87	84	97
24	117	115	98	105	84	80

Table 1: Reanalysis after 4-fold predilution in FIX deficient plasma of 5 plasmas with strong LA activity (dRVVT > 1.7; SCT > 2.6). Results expressed as % FIX activity. Mean of 2 replicates for each dilution and method.

	Rox Factor IX		Actin FS		PTT-LA		
Sample no	Dil 1:80	Dil 1:160	Dil 1:10	Dil 1:20	Dil 1:5	Dil 1:10	Dil 1:20
14	113	113	84	89	69	81	92
17	127	137	91	96	61	82	104
19	112	112	127	122	72	91	113
22	94	93	109	91	8	21	60
25	93	88	104	90	not detectable	not detectable	not detectable

Table 2: Assigned FIX potencies (%) at different dilutions using Rox Factor IX, Actin FS and PTT-LA.

✉ Pia.Bryngelhed@rossix.com