P241

Dilutional linearity demonstrated over the whole measuring range for two extended half life recombinant Factor IX (EHL-rFIX) products with a chromogenic Factor IX method.

Pia Bryngelhed, Per Rosén (Rossix AB, Molndal, Sweden)

INTRODUCTION

Analysis of EHL-rFIX products has shown large method discrepancies within one-stage (OS) methods and vs chromogenic substrate (CS) methods. For some methods, the discrepancy is more pronounced at low FIX activities. Adherence to dilutional linearity is an important parameter in assessing method performance.

AIM

The purpose of this work was to investigate dilutional linearity on analysis of two EHL-rFIX products, one **glycoPEGylated** [Nonacog beta pegol; N9-GP (Refixia/Rebinyn), Novo Nordisk] and one **fused with albumin** [albutrepenonakog alfa (Idelvion), CSL Behring], using a chromogenic Factor IX method (Rox Factor IX).

CONCLUSIONS

- Dilutional linearity for glycoPEGylated EHL-rFIX (Nonacog beta pegol) was obtained in the tested range 0.2 – 200% (0.002 – 2.0 IU/mL) when applying Rox Factor IX on ACL TOP 500 and STA-R Evolution.
- Dilutional linearity for albumin fused EHL-rFIX (albutrepenonakog alfa) was obtained in the tested range 1.7 – 165% (0.017 – 1.65 IU/mL) when applying Rox Factor IX on ACL TOP 500 and STA-R Evolution.
- There is a good agreement in results obtained using the SSC/ISTH Secondary Coagulation Standard Lot No 4 (plasma) or the 5th International Standard (IS) hFIX Concentrate as calibrators.

MATERIALS AND METHODS

FIX doses were prepared in FIX deficient plasma (Congenital FIX deficient plasma, Stago) in the range 0.2 – 200% (Nonacog beta pegol, NovoNordisk) and 1.7 – 165% (albutrepenonakog alfa, CSL Behring) based on potency assignments obtained using the chromogenic Factor IX method, Rox Factor IX (Rossix AB). Two independent assay series were run with Rox Factor IX on both ACL TOP 500 (Instrumentation Laboratories) and on STA-R Evolution (Stago). The 5th International Standard (IS) Human Blood Factor IX Concentrate 14/148 and the SSC/ISTH Secondary Coagulation Standard Lot No 4 (plasma) (both from NIBSC, UK) were used as calibrators.

RESULTS

For both EHL-rFIX sources, and irrespective of choice of calibrator, dilutional linearity was demonstrated over the whole measuring range. The r^2 values were > 0.99 on both ACL TOP 500 and STA-R Evolution and with no trend towards deviations from linearity, especially at low FIX activites. The results obtained using the 5th IS or the SSC#4 as calibrator were in good agreement with correlation slopes ranging from 0.95-0.98, r^2 > 0.999 for both EHL-rFIX products on both instruments.

TABLE 1, FIX, % for Nonacog beta pegol (Refixia/Rebinyn) calculated vs SSC#4 (plasma)

Expected activity (FIX, %):	200	170	150	100	50	20	10	5	2	0,5	0,2
ACL TOP	215	181	154	101	51	19	9	5,4	2,3	0,4	0,2
STA-R Evolution	198	156	139	97	48	20	12	4,5	2,2	0,4	0,2

TABLE 2, FIX, % for Nonacog beta pegol (Refixia/Rebinyn) calculated vs 5th IS (concentrate)

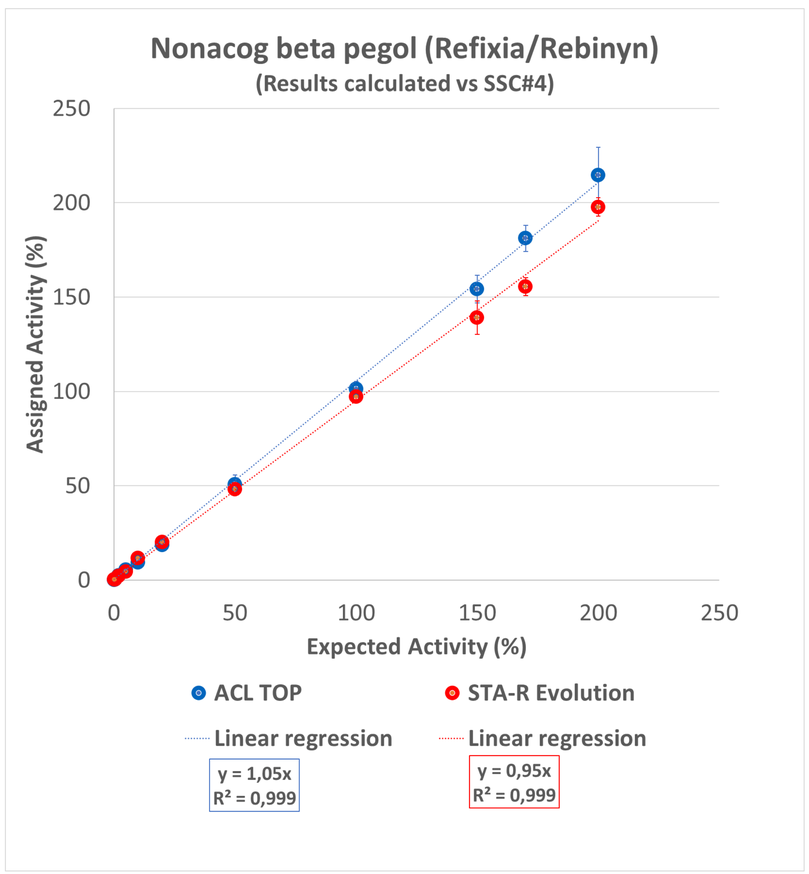
Expected activity (FIX, %):	200	170	150	100	50	20	10	5	2	0,5	0,2
ACL TOP	204	170	150	94	46	17	9	5,1	2,3	0,4	0,1
STA-R Evolution	192	152	136	94	47	20	11	6,1	1,7	0,7	0,4

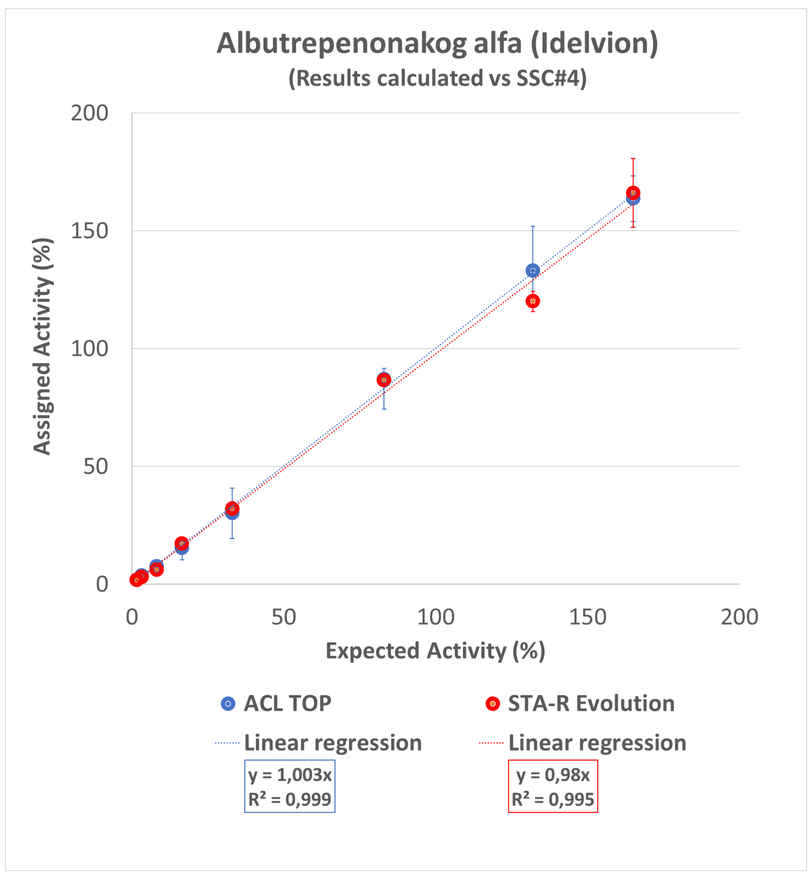
TABLE 3, FIX, % for Albutrepenonakog alfa (Idelvion) calculated vs SSC#4 (plasma)

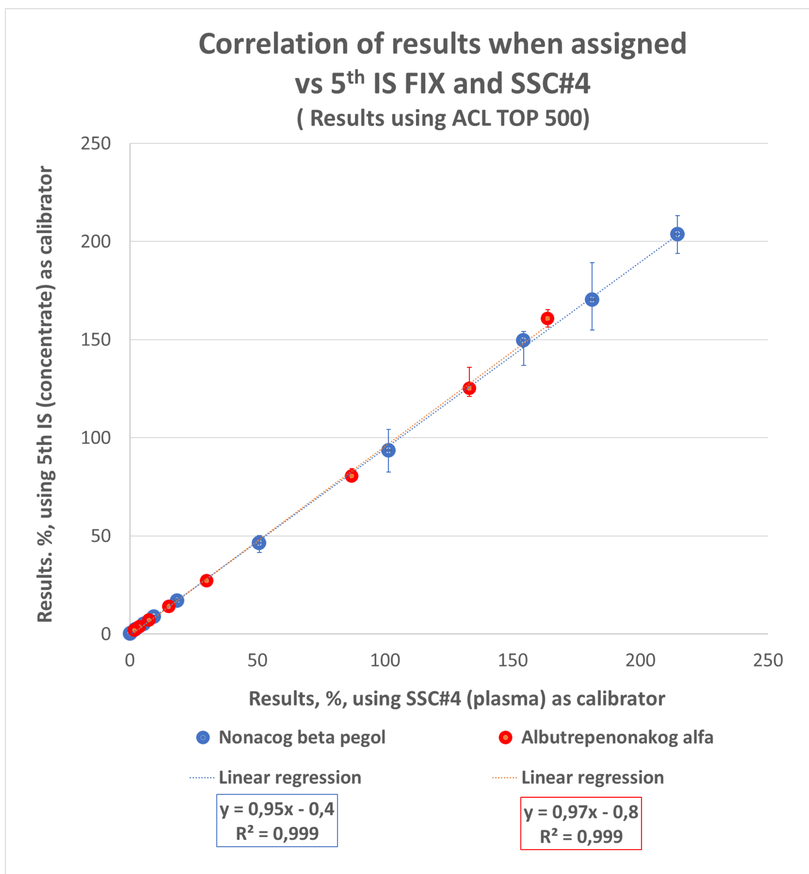
Expected activity (FIX, %):	165	132	83	33	16,5	8,3	3,3	1,7
ACL TOP	164	133	87	30	15	7,5	3,7	1,8
STA-R Evolution	166	120	87	32	17	6,2	3,1	1,7

TABLE 4, FIX, % for Albutrepenonakog alfa (Idelvion) calculated vs 5th IS (concentrate)

Expected activity (FIX, %):	165	132	83	33	16,5	8,3	3,3	1,7
ACL TOP	161	125	80	27	14	7,2	3,7	1,8
STA-R Evolution	162	118	80	31	17	6,0	2,5	1,4







REFERENCES

Kitchen S, Gray E, Mertens K. Monitoring of modified factor VIII and IX products. *Haemophilia 20 (Suppl. 4), 36-42 (2014)*.

Tiefenbacher et al. Qualification of a select one-stage activated partial thromboplastin time-based clotting assay and two chromogenic assays for the post-administration monitoring of nonacog beta pegol. *J Thromb Haemost 15, 1901-1912 (2017)*.

EAHAD 2019
12th ANNUAL CONGRESS OF THE EUROPEAN ASSOCIATION FOR HAEMOPHILIA AND ALLIED DISORDERS
PRAGUE CZECH REPUBLIC
6-8 February 2019