The performance of FVIII and FIX measurement in ECAT surveys

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Introduction

The measurement of Factor VIII (FVIII) and Factor IX (FIX) clotting activity is used for both the diagnosis of haemophilia A or B and the monitoring of treatment and therefore requires precise laboratory measurement. We evaluated the performance of FVIII and FIX measurement in surveys of the ECAT external quality assessment programme.

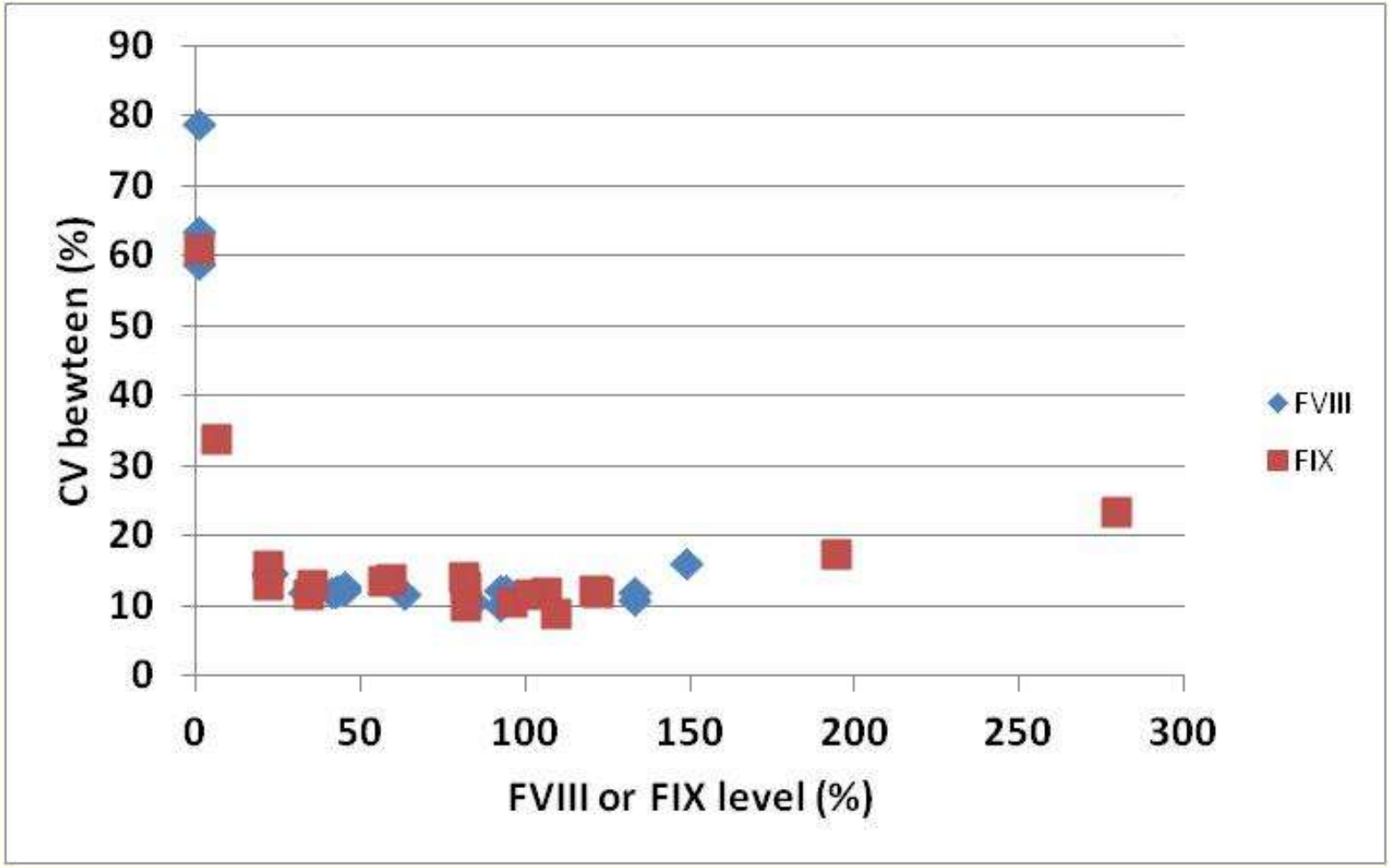
Methods

- **Test results of FVIII and FIX of 175 - 215 participants were evaluated for the period 2010 – 2012.**

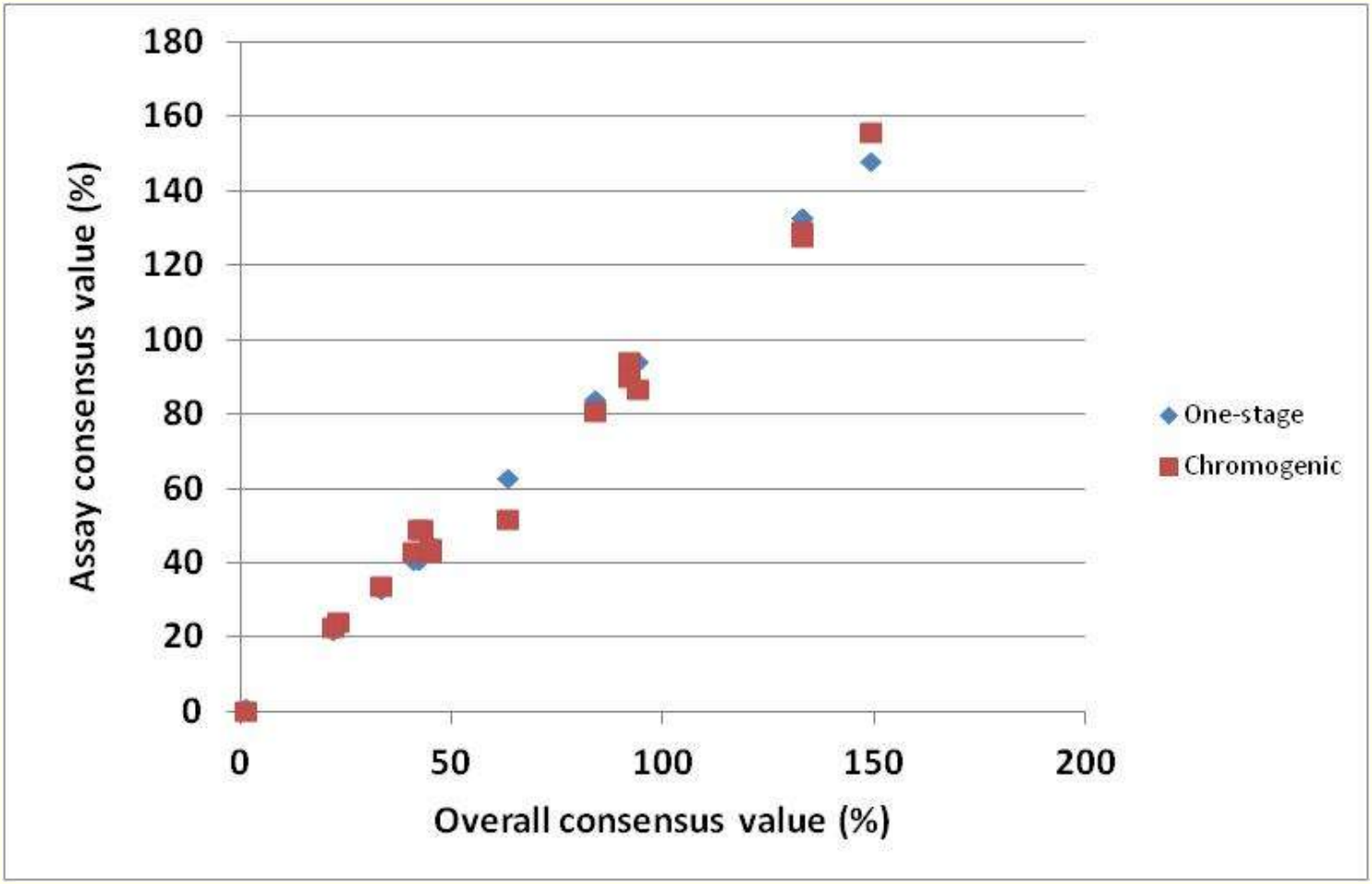
- **The between-laboratory variation (BCV) was assessed at different Factor VIII levels.**

- **The long-term analytical coefficient of variation (LCVa) was assessed using a linear regression model (P. Meijer *et al*. Clin Chem 2002;48:1011-15). The LCVa is a measure for the long-term analytical performance of a laboratory.**

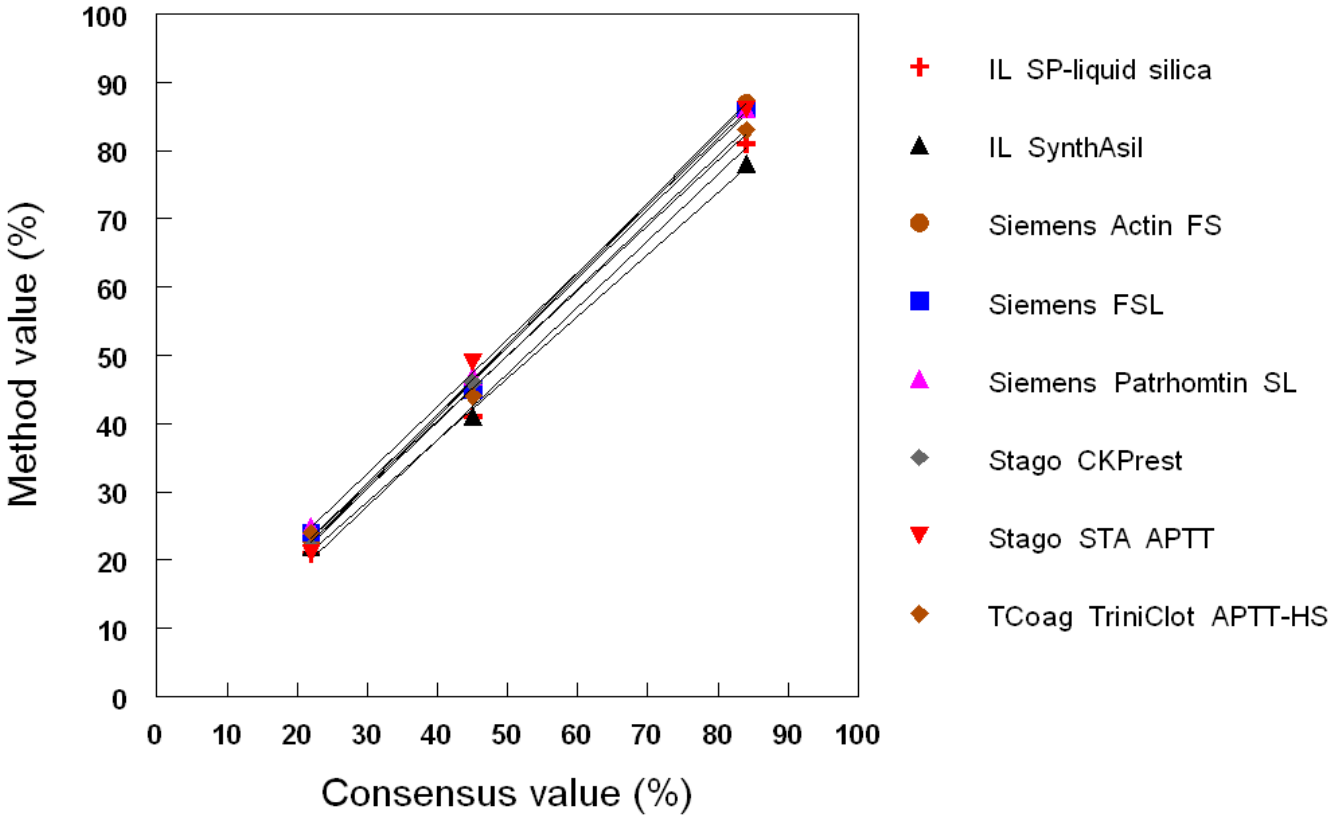
Results FVIII + FIX



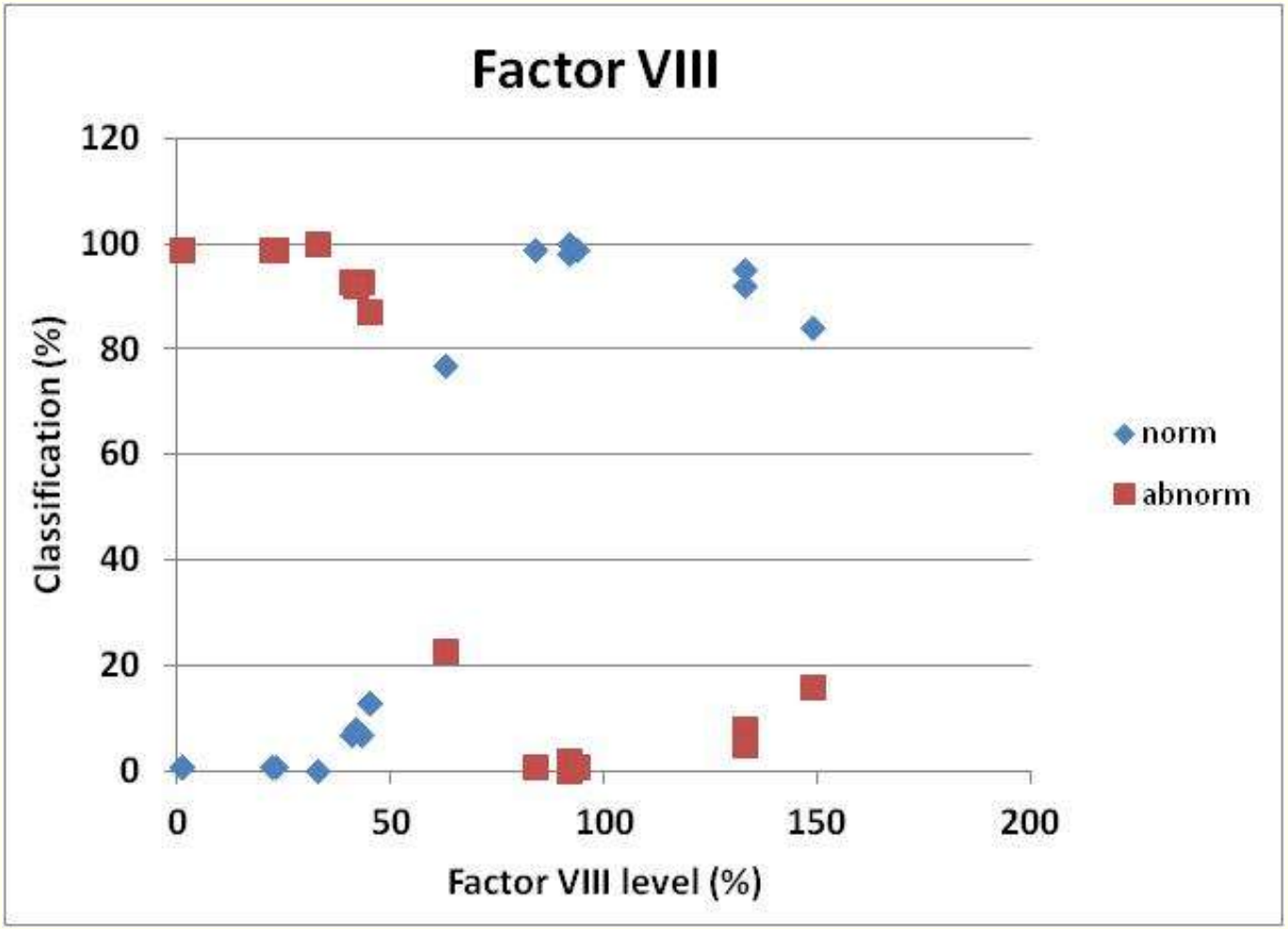
Results FVIII



Results FVIII



Results FVIII



Results FVIII

Severe Haemophilia A

N = 201

98x < [value] :

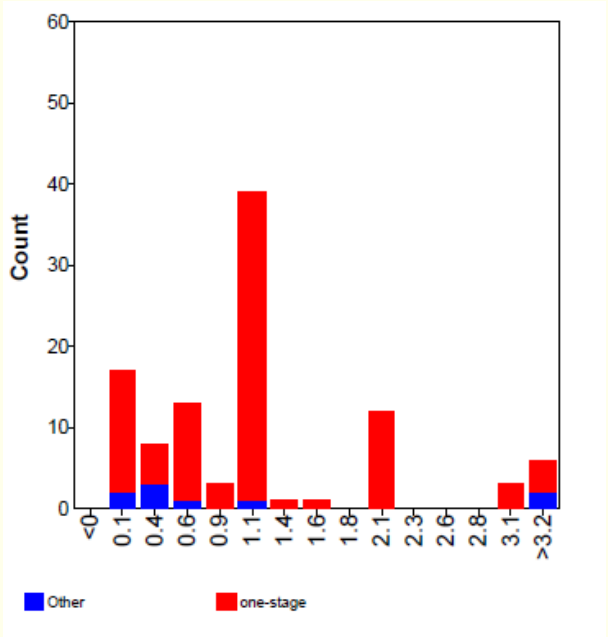
16 x < [0.1 – 0.9]
69 x < 1
13 x < [2 – 12] (13%)

103 numerical results

Robust mean	**:**	**0.95%**
Median	**:**	**1.00%**
SD	**:**	**0.75%**
Range	**:**	**0 – 41%**

Result > 1% : 22 (21%)

Overall: 17% reported a result > 1% FVIII



Discussion

Table 1. FVIII:C activity assay with one-stage method of five samples from severe haemophilia A patients (1, 5, 7, 8 and10) and five samples from moderate haemophilia A patients (2, 3, 4, 6 and 9). Eleven different aPTT reagents were used in the FVIII:C assays.

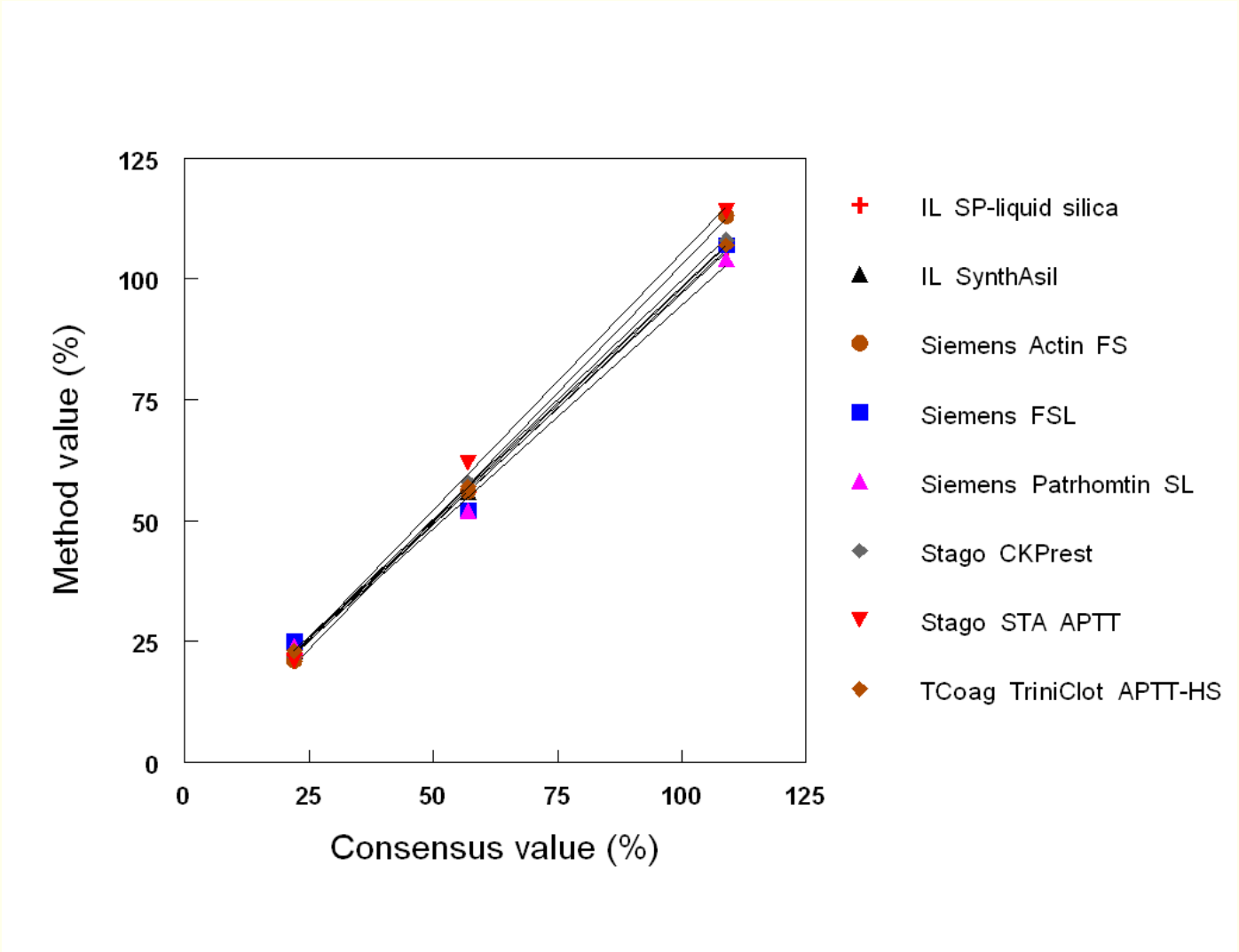
		Reagents										
Sample	Mutation gene type	1	2	3	4	5	6	7	8	9	10	11
1	inv. Intron 1	1.4	0.4	0.4	0.3	0.9	0.1	0.3	0.8	1.4	0.3	0.5
2	ex10 Tyr 476 stop C → G	6.4	4.1	5.1	4.3	5.4	3.8	4.6	4.6	6.8	5.2	5.8
3	ex7 Hs256Arg A → G	2.5	1.4	1.5	1.4	2.1	1.2	1.3	1.9	3.6	1.7	1.8
4	ex14 Asp121461u C → G	5.4	3.9	4.5	3.7	5.0	3.8	4.1	4.6	8.3	5.0	4.9
5	inv. Intron 22	1.3	0.4	0.4	0.4	0.9	0.1	0.2	0.8	2.0	0.4	0.4
6	ex23 Arg2163Cys C → T	1.8	0.8	0.9	0.9	1.3	0.5	0.8	1.5	3.0	1.0	1.0
7	ex14 Arg795stop C → T	nd	0.4	0.7	0.3	0.6	0.1	0.3	0.9	1.3	nd	0.5
8	inv. Intron 1	1.7	1.0	1.0	0.8	0.9	0.5	0.6	0.9	1.8	0.7	0.9
9	ex7 His256Arg A → G	2.8	1.7	2.0	1.3	3.0	1.6	1.9	2.9	1.9	2.1	2.6
10	inv. Intron 22+/−	2.1	1.1	0.4	1.0	1.9	1.0	1.3	2.0	1.6	1.2	1.6

The results of the assays are expressed as IU dL^{-1}.

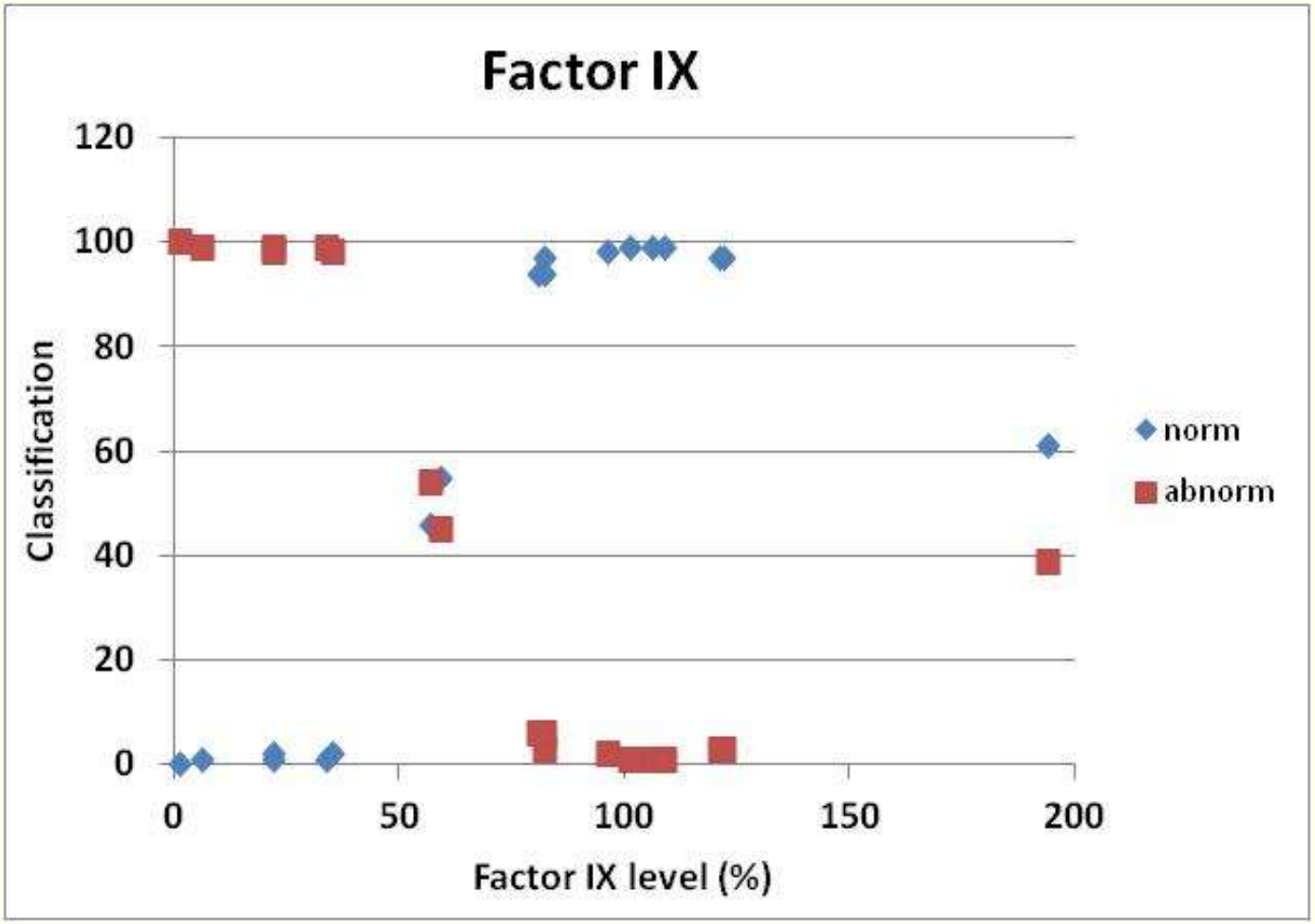
Reagents: 1, STA Cephascreen (Stago); 2, STA APTT Kaolin(Stago); 3, PTT-LA (Stago); 4, PTT a (Stago); 5, PTT Reagent (Stago); 6, Synthasil (HemosIL); 7, APTT-SP (HemosIL); 8, Dade® Actin® FSL Activated PTT Reagent (Dade Behring); 9, Dade® Actin® FS Activated PTT Reagent (Dade Behring); 10, Pathromtin SL (Dade Behring); 11, Platelin® LS (Biomerieux); NO, Not Determined.

Haemophilia (2008), 14 (Suppl. 3), 76–82

Results FIX


125
100
75
50
25
0
Method value (%)
0
25
50
75
100
125
Consensus value (%)
IL SP-liquid silica
IL SynthAsil
Siemens Actin FS
Siemens FSL
Siemens Patrhomtin SL
Stago CKPrest
Stago STA APTT
TCoag TriniClot APTT-HS

Results FIX


Factor IX
120
100
80
60
40
20
0
Classification
0
50
100
150
200
Factor IX level (%)
norm
abnorm

Results FIX

Severe Haemophilia B

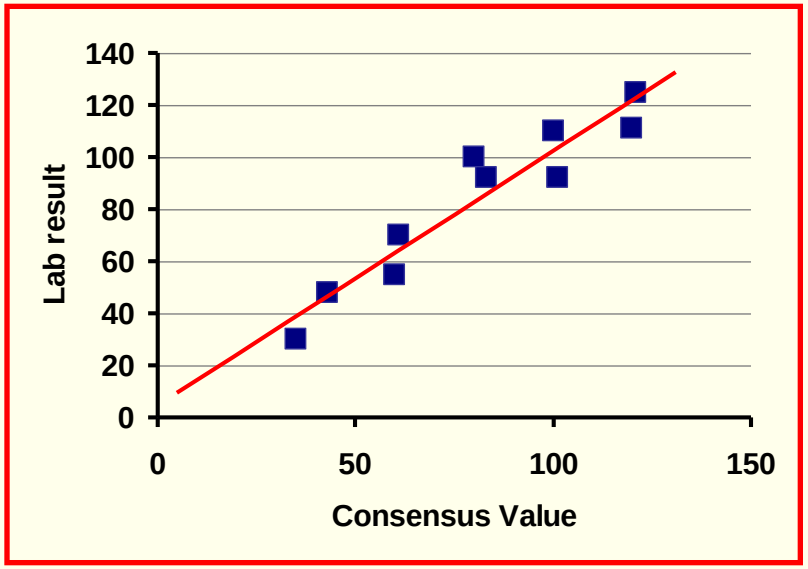
Factor IX (plasma 10.60)	n	mean	CV	range
total group	154	1.1	61.1%	0.1 – 4.3
One-stage Clotting Assay	152	1.1	61.1%	0.1 – 4.3

Activator	n	mean	CV	Range
HemosIL APTT-SP liq. sil. (IL)	10	1.0	18.2%	0.5 – 1.3
HemosIL Synthasil (IL)	20	1.0	47.6%	0.3 – 2.0
Actin FS (Siemens)	16	1.1	45.9%	0.8 – 3.0
Actin FSL (Siemens)	16	1.0	42.3%	0.2 – 2.0
Pathromtin SL (Siemens)	20	1.1	103%	0.1 – 4.3
PTT Automate./STA APTT (Stago)	24	1.1	56.9%	0.3 – 3.0
Ceph./Kaolin - CKPrest (Stago)	15	1.0	58.3%	0.1 – 3.0

18x < [value]

Numerical results : 29 x result > 1% (19%)

Long-term evaluation



IMPRECISION

$$LCV_a = \frac{s_{y|x}/b}{\bar{X}} \bullet 100\%$$

BIAS

$$B = \frac{\sqrt{\frac{n-1}{n} \bullet (b-1)^2 \bullet s_x^2 + (\bar{Y} - \bar{X})^2}}{\bar{X}} \bullet 100\%$$

X = consensus value ; $\bar{X}$ = mean value for X.

s_x = standard error of X

Y = laboratory value ; $\bar{Y}$ = mean value for Y.

b = slope

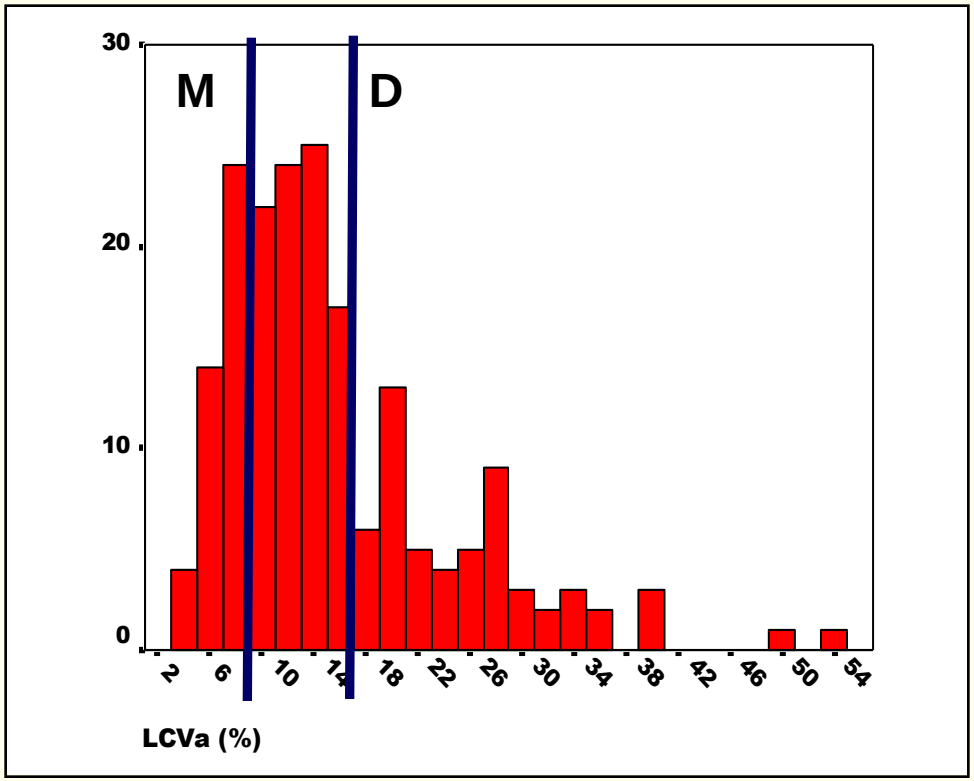
$s_{y|x}$ = variability of the regression line, which is calculated based on the least-square method.

n = number of laboratory results

TOTAL ERROR

$$TE = \frac{\sqrt{s_{y|x}^2 + (b-1)^2 \bullet s_x^2 + (\bar{Y} - \bar{X})^2}}{\bar{X}} \bullet 100\%$$

Long-term evaluation FVIII



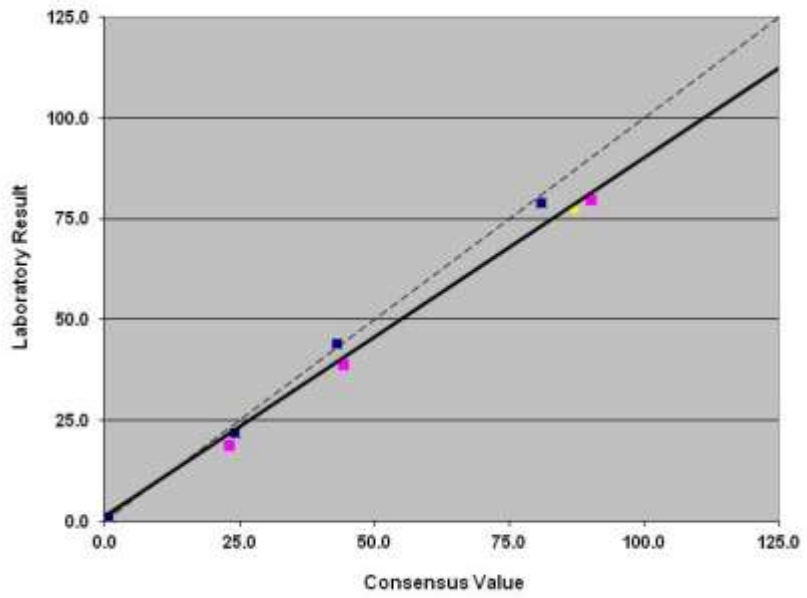
	Performance goals based on the biological variation	
	Diagnosis	Monitoring
Factor VIII	17.5	8.3
% Pass	71%	16%

Descriptive statistics

N : 187
Mean : 15.7%
Median : 13.3%
Range : 5.1 – 39.9%
(2.5 – 97.5 CI)

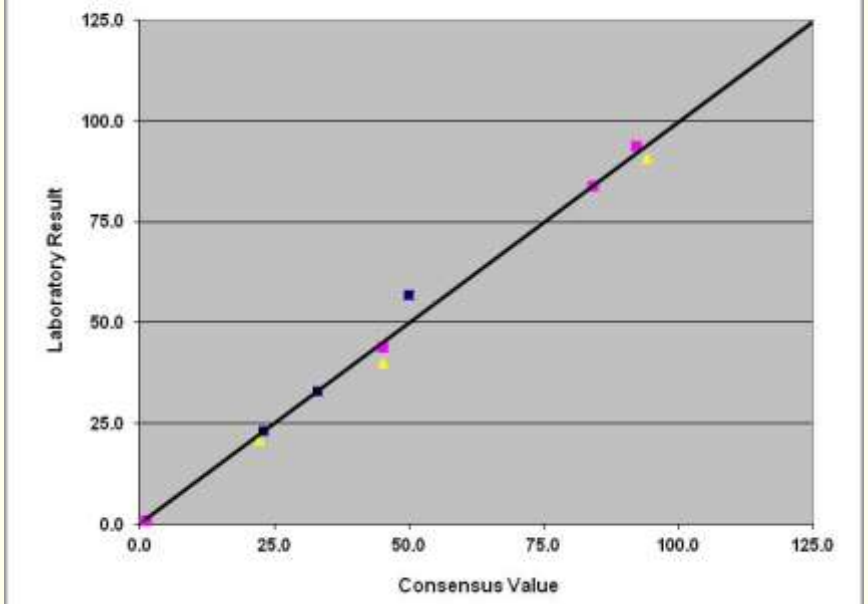
Long-term evaluation FVIII

Chromogenic



Long-term CVanalytical	5.8%
Bias	12.1%

Clotting



Long-term CVanalytical	5.6%
Bias	0.5%

SIX-SIGMA

Sigma = (TEa – Bias) / CV

$$TEa < 1.65 \times (0.5 \times CV_{ws}) + 0{,}25 \times (CV_{ws}^2 + CV_{bs}^2)^{1/2}$$

Factor VIII

TEa = 13.3% (Westgard database – minimum specs)

Chromogenic	Clotting
Bias = 12.1%	Bias = 0.5%
CV = 5.8%	CV = 5.6%
Sigma = 0.21	Sigma = 2.29

Conclusions

- The between-laboratory variation for FVIII and FIX is comparable.
- Differences between reagents are small.
- The accuracy of measurement in the low range needs improvement.
- The long-term performance evaluation may help laboratories in their quality management.
- Especially for monitoring ttreatment a more stable performance over time is needed.