Evaluation of single-site and multisite precision of factor IX measurement

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BACKGROUND

Precision and accuracy of factor IX determination is of highest importance to properly screen, diagnose and manage haemophilia B patients. Accuracy can be ensured through proper activity assignment and link to international standards. Precision rely mostly on the properties of the assay methodology used (combination of reagents, instrument and data acquisition).

AIMS

We evaluated single-site and multisite precision of FIX measurement with **STA®-ImmunoDef IX** and **STA®-C.K. Prest®** reagents, following EP05-A3 CLSI guidelines recommendations (1).

MATERIALS & METHODS

Samples used were 5 native individual samples spanning the assay measuring range.
They were tested with 3 lots of **STA®-ImmunoDef IX** and 1 unique lot for all other reagents.
For single-site precision, a total of 20 days were performed, with 2 runs per day and all tests measured in duplicates, on both **STA R®** and **STA Compact®**. Multisite precision was determined through experiments on 3 sites, 5 runs per site (on 5 different, non-consecutive days), 5 replicates per run, on **STA R®** only.
The studies were performed at Amarok Biotechnologies (Saint-Malo, France), Laboratoire Cerba (Saint-Ouen-L'Aumône, France) and Medical University of South-Carolina (Charleston, USA).
All reagents and instruments were from Stago, Asnières-sur-Seine, France.

RESULTS

Checking results with Grubbs' test, less than 1% of them were outliers and all sites kept the calibration curve of day 1 to determine FIX levels along the whole experiment. For sample 1, an additional point was included in the calibration curve and used only if needed (some very low results were otherwise out of range).
Results were calculated utilizing ANOVA tests.

Sample	N	Mean (%)	Repeatability		Within-Lab precision	
			SD (%)	%CV	SD (%)	%CV
1	240	0.6	0.07	11.7	0.14	23.3
2	240	8.4	0.46	5.5	0.89	10.6
3	240	60.4	1.74	2.9	4.60	7.6
4	240	122.5	3.33	2.7	8.25	6.7
5	240	211.4	16.30	7.7	24.52	11.6

Table 1: Single site precision (STA R®) – pooled results from 3 lots

Sample	N	Mean (%)	Repeatability		Within-Lab precision	
			SD (%)	%CV	SD (%)	%CV
1	240	0.8	0.05	6.3	0.15	18.8
2	240	9.6	0.43	4.5	0.84	8.8
3	240	58.5	2.20	3.8	3.47	5.9
4	240	115.5	3.72	3.2	5.65	4.9
5	240	199.7	8.46	4.2	15.72	7.9

Table 2: Single site precision (STA Compact®) – pooled results from 3 lots

Sample	N	Mean (%)	Repeatability		Between sites precision		Reproducibility	
			SD (%)	%CV	SD (%)	%CV	SD (%)	%CV
1	225	0.7	0.07	10.0	0.13	18.6	0.17	24.3
2	225	9.2	0.39	4.2	0.75	8.2	1.05	11.4
3	225	59.4	2.00	3.4	1.63	2.7	4.08	6.9
4	225	120.6	3.72	3.1	5.08	4.2	8.30	6.9
5	225	217.9	10.07	4.6	0.00	0.0	17.12	7.9

Table 3: Multisite precision (STA R®) – pooled results from 3 sites and 3 lots/site

CONCLUSION

FIX measurement with STA®-ImmunoDef IX and STA®-C.K. Prest® is very precise and reproducible both single-site and multisite, **even at low FIX levels, without the need for any recalibration**.
When considering less variables than this full precision study, serial dilutions of FIX showed that **down to 0.7%** FIX, CVs observed on 8 replicates over 5 days on the same instrument did not exceed 14.7%.

References:
(1) CLSI *Evaluation of Precision of Quantitative Measurement Procedures, Approved guideline – third edition.* CLSI document EP05-A3 Wayne, PA: Clinical and Laboratory Standard Institute, 2014