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

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BACKGROUND

Precision and accuracy of factor VIII determination is of highest importance to properly screen, diagnose and manage haemophilia A 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 FVIII measurement with **STA®-ImmunoDef VIII** 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 VIII** 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 FVIII levels along the whole experiment.
Results were calculated utilizing ANOVA tests.

Sample	N	Mean (%)	Repeatability		Within-Lab precision	
			SD (%)	%CV	SD (%)	%CV
1	240	2.0	0.08	4.0	0.22	11.0
2	240	8.6	0.26	3.0	0.84	9.8
3	240	54.0	1.47	2.7	4.95	9.2
4	240	111.9	2.93	2.6	7.74	6.9
5	240	342.7	16.61	4.8	24.17	7.1

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	2.1	0.10	4.8	0.21	10.0
2	240	8.7	0.36	4.1	0.66	7.6
3	240	51.1	1.88	3.7	3.83	7.5
4	240	100.4	2.87	2.9	6.76	6.7
5	240	322.1	12.43	3.9	21.87	6.8

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	1.9	0.10	5.3	0.12	6.3	0.22	11.6
2	225	8.4	0.35	4.2	0.57	6.8	0.79	9.4
3	225	50.9	1.64	3.2	2.54	5.0	4.23	8.3
4	225	106.6	2.84	2.7	9.50	8.9	11.38	10.7
5	225	338.4	10.07	3.0	16.34	4.8	24.42	7.2

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

CONCLUSION

FVIII measurement with STA®-ImmunoDef VIII and STA®-C.K. Prest® is very precise and reproducible both single-site and multisite, **even at low FVIII levels, without the need for any recalibration.**
Additional results from serial dilutions of FVIII showed that **down to 0.7% FVIII**, CVs observed on 8 replicates over 5 days did not exceed 13.8%.

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

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