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Standardization and Automation of Thrombin Generation Assay

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the cone-plate method. We evaluated the analytical performance of the ZL 6000i to measure WBV.

Aims: We established a reference range and validated precision using quality control (QC) and human blood samples. Storage time and temperature effects were also evaluated.

Methods: Samples were collected from 287 normal adults (162 males and 125 females) to establish a reference range. We evaluated total precision for 20 days using QC viscosity materials at shear rates of 1, 50, and 200 s⁻¹, and within-run precision using phlebotomy subjects at shear rates of 1, 5, 30, and 200 s⁻¹. Aliquots of the phlebotomy samples were stored at room temperature and 5°C, respectively, for the stability analysis. All samples were tested at baseline (control) and 6, 24, 48, 72, and 96 hours later to assess changes over time.

Results: Reference intervals differed between males and females (Table 1).

TABLE 1 Reference intervals for specific shear rates in health men and women

	Male (n = 162)		Female (n = 125)	
Age (years)	43 ± 10.9		46 ± 11.0	
Hct (%)	46.2 ± 2.63		40.5 ± 2.29	
Shear rates	Median WBV	Central 95 percentile RI	Median WBV	Central 95 percentile RI
1	21.88	16.554-36.248	17.44	12.168-37.303
5	9.48	7.561-12.847	7.69	6.058-13.172
30	5.35	4.311-6.369	4.51	3.582-5.915
200	3.93	3.261-4.643	3.38	2.816-3.960

The coefficients of variation (CVs) for within-run and total precision with QC material and human whole blood were < 7.5% at all shear rates. The CVs for between-day precision with QC material were < 2.5% at all shear rates. WBV of the samples stored at room temperature for up to 6 hours and at 5°C for up to 2 days did not change compared to the control.

Conclusions: We recommend that separate reference intervals be used for men and women. The results indicate that the ZL-6000i provides rapid, accurate and reproducible WBV data. Viscosity measurements should be finished within 6 hours after sample collection, and samples that cannot be measured within the 6-hour window should be refrigerated until ready to test.

TABLE 1 Performances of ST-Genesia on reference, hypo- and normo-coagulable plasma

n=41	Sample 1 = STG - QualiTest Norm DS			Sample 2 = STG - QualiTest Low DS			Sample 3 = STG - RefPlasma DS		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Lag time (min)	0,92	0,04	4,6%	1,18	0,05	4,1%	0,98	0,05	5,2%
Peak (nM)	501,92	16,17	3,2%	206,80	10,12	4,9%	486,25	16,50	3,4%
Time to Peak (min)	1,97	0,07	3,6%	2,32	0,10	4,3%	2,17	0,08	3,5%
ETP (nM.min)	1602,66	65,24	4,1%	522,55	20,55	3,9%	1740,53	67,63	3,9%
Velocity Index (nM/min)	718,69	55,87	7,8%	244,02	23,37	9,6%	598,74	49,27	8,2%

PB 434 | Standardization and Automation of Thrombin Generation Assay: on the Way to the Clinical Lab

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Background: Thrombin generation (TG) is known for more than 60 years. Several developments have been done through the years to improve its usability but it remains a research use tool because of a lack of standardization of methods. Typical inter-day precision of TG assays is around 10 to 30% depending on the parameter analyzed. It depends on the concentration and the source of tissue factor (TF) in the reagent, the use of external or local normal plasma to normalize the results, the operator as well as the method (1-2).

Aims: ST Genesia, a new analyzer intended to measure thrombin generation in a fully automated way, was evaluated in our lab for validation purposes.

Aside from biological outcomes of our protocol in the anticoagulant treatment setting, the purpose of this evaluation was also to determine how precise could become TG measurement.

Methods: 41 independent runs of measurement were performed with the same batch of STG - DrugScreen on ST Genesia. On each run, 3 freeze-dried samples were tested prior to testing fresh or frozen patient samples. 2 of these samples were internal quality control samples (hypocoagulable and normocoagulable) and 1 is intended to be used as reference plasma for normalizing results (2).

Results: Mean, standard deviation and coefficient of variation achieved are reported in table 1.

Conclusions: Automation, enhanced control of temperature throughout the assay and standardization of thrombin generation measurement help to achieve highly reproducible results, first step to introduce this assay in the clinical lab.

References:

- (1) Dargaud Y et al *Evaluation of a standardized protocol for thrombin generation measurement using the calibrated automated thrombogram: An international multicentre study* Thromb Res 2012; 130(6): 929-934.
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