Standardisation and automation of thrombin generation assay: TG on its way to the clinical lab for haemophilia patients

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BACKGROUND

Currently, Thrombin Generation (TG) is a labour intensive and poorly standardised technique used in few specialist centres. In such centres, TG is useful to monitor the efficacy of replacement therapy in patients with haemophilia (Dargaud et al, Blood 2010). In addition, TG has shown improved capacity to evaluate an individual's bleeding tendancy compared to factor levels measured using traditional assays (Trossaert et al Haemophilia 2014). Despite this, multicenter studies of the clinical utility of this assay have been limited by poor standardisation of the method.

AIMS

The ST Genesia, a new fully automated analyser intended to measure TG, was evaluated in our laboratories to validate the STG-BleedScreen reagent, utilising a trigger containing a low concentration of tissue factor (TF).
Demonstration of acceptable bi-centric precision and accuracy was intended to validate analytical utility of the assay in the clinical studies performed later.

MATERIALS AND METHODS

Each of the 2 centers assessed the same lot of STG-BleedScreen reagent on the ST Genesia analyser (both from Stago, Asnières sur seine, France) to assay patient plasma.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 (IQC) samples (one hypocoagulable and one normal) plus one intended to be used as reference plasma for normalising results as per the protocol in (Perrin J et al, Thromb Res 2015).

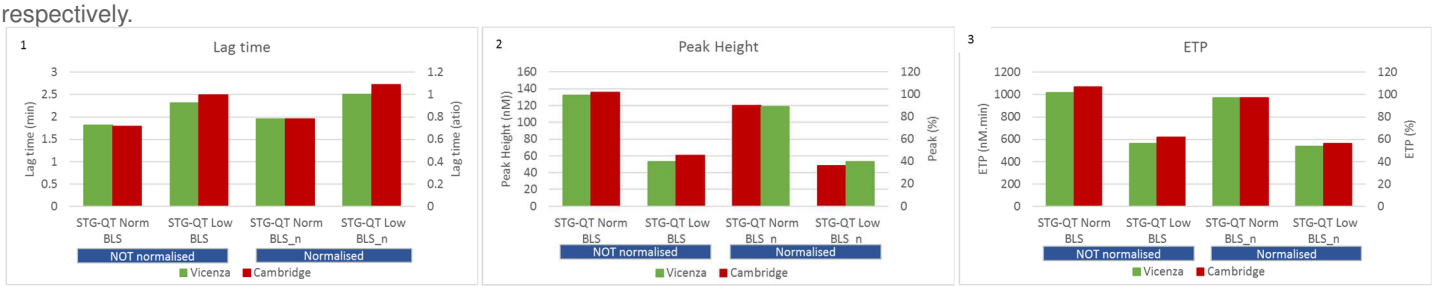
RESULTS

Precision across the 65 independent runs (27 runs on one site, 38 on the second) demonstrated good performance across all parameters. Initial results without normalisation showed CV results below 8% for all parameters except Velocity Index (VI) using the STG-QualiTest Norm BLS and below 21.4 % with STG-QualiTest Low BLS again with the exception of the VI (data not shown). Precision was improved by normalisation with the reference plasma resulting in the CVs displayed in Table 1 below.

		Lag time (ratio)	Peak Height (%)	Time to Peak (ratio)	ETP (%)	Velocity Index (%)	StartTail (ratio)
STG-QualiTest Norm BLS	n	65	65	65	65	65	65
	Mean	0.78	89.40	0.92	97.21	92.43	1.10
	SD	0.05	6.04	0.02	4.01	9.84	0.06
	CV	**5.9%**	**6.8%**	**2.6%**	**4.1%**	**10.6%**	**5.4%**
STG-QualiTest Low BLS	n	65	65	65	65	65	65
	Mean	1.05	38.28	1.30	55.09	28.24	1.41
	SD	0.07	3.11	0.04	2.39	4.99	0.07
	CV	**6.9%**	**8.1%**	**2.7%**	**4.3%**	**17.7%**	**5.2%**

Table 1: Inter-run precision (normalised results only) over 2 sites.

Across both centres, relative deviations of Lag time, Peak height and ETP were lower than 5.1% on STG-QualiTest Norm BLS and lower than 12.1% on STG-QualiTest Low BLS before normalisation of results. After normalisation these were lower than 1% and 9% respectively.



Graphs: mean values of STG-QualiTest BLS plasmas for Lag time (1), Peak Height (2) and ETP (3), without and with normalisation with STG-RefPlasma BLS on each site.

CONCLUSION

Automation, enhanced control of temperature throughout the assay and normalisation of results help to achieve **good comparability and reproducibility of results when testing on two different sites and at low TF concentration.**
This enhanced standardisation of TG was a pre-requisite to introduce the assay in the clinical lab.
A subsequent clinical study is now ongoing.

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