

Diagnostica di laboratorio in emostasi e trombosi

VALIDATION OF THROMBIN GENERATION MEASUREMENT IN PLATELET- RICH-PLASMA: EVOLUTION IN THE ST GENESIA® SYSTEM

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Background and Aims. Thrombin generation assay (TGA) represents a promising tool for monitoring novel non-replacement therapies in haemophilia. ST Genesia® system (*Stago, Asnières-sur-Seine, France*) is validated to measure thrombin generation (TG) in platelet poor plasma (PPP) but there is no standardized method available on this platform for testing TG in platelet-rich plasma (PRP). Such a method would enable the investigation of platelet–drug interactions and provide a more comprehensive assessment of the patient's haemostatic function.The aim of this study was to experiment and validate, on ST Genesia system, the technical reliability of TG measurement in PRP, evaluating: intra- and inter-run reproducibility, PRP sample stability over time, effectiveness of normalization by reference plasma, absence of methodological interference from the platelet component, and robustness of the method.

Methods. The procedure required the following materials: PRP Reagent (CAT PRP-Reagent, Stago) that contains relipidated Tissue Factor that allows the initiation of reaction, a home made PPP reference plasma (RefPlasma) prepared from a pool of at least 20 healthy donors, used for result normalisation, analytical variability monitoring, and internal system calibration and the PRP samples under investigation. RefPlasma validation was performed by calculating the intra-run (≥10 replicates) and inter-run (≥3 days) coefficients of variation (CV%), alongside assessment of routine coagulation parameters (PT, aPTT, fibrinogen, AT). PRP sample stability was evaluated by performing TG measurements at three time points: T0 (baseline), T1 (1 hour post T0), and T2 (3 hours post T0).

Results. RefPlasma validation demonstrated acceptable intra-run CV% (10 replicates within a single run) and Inter-run CV% (across 3 runs on separate days) described in Table 1. Routine coagulation parameters (PT, aPTT, fibrinogen, AT) were within normal reference ranges. PRP stability testing revealed a progressive decline in Peak Height, ETP, and Velocity Index from T0 through T2, accompanied by a corresponding increase in Lag Time, Time to Peak, and Start Tail.

Conclusions. With coefficients of variation predominantly below 10%, the RefPlasma demonstrated satisfactory reproducibility both within individual runs and across runs performed on different days. Regarding PRP stability, the progressive reduction of TG parameters (Peak Height, ETP) over time suggests that specimens should be analysed within 3 hours of collection to mitigate pre-analytical degradation. This finding warrants further investigation, particularly concerning the feasibility of test centralisation when processing samples received from external centres. In conclusion, this procedure represents a validated approach for TGA in PRP, enabling a more physiologically relevant evaluation when it is important to have a global assessment of drug-coagulation interactions.

Parameter	CV% INTRA-RUN	CV% INTER-RUN
Lag Time	2.63%	11.74%
Peak Height	2.37%	3.43%
Time to Peak	2.89%	8.07%
ETP	2.65%	2.37%
Vel.Index	6.02%	8.69%
Start Tail	2.91%	5.81%