Thrombin generation assay (CAT)

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Abstract

TG in plasma was measured with the calibrated automated thrombogram (CAT) assay as developed by Hemker and co-workers [18-20]. Briefly, 80 µl platelet poor plasma (PPP) was mixed with 20 µl of a mixture containing tissue factor (Dade-Behring) at a final concentration of 1 pM and phospholipid vesicles (f.c. 4 µM 20 mol% phosphatidylserine, 60 mol% phosphatidylcholine and 20 mol% phosphatidyl-ethanolamine, Avanti). To calibrator wells, 20 µl of calibrator (α2macroglobulin- thrombin complex, [19]) was added instead of TF and PL. After 10 minutes of incubation at 37°C, thrombin generation was initiated by the addition of 20 µl of the thrombin specific substrate, Z- Gly-Gly-Arg-7-amino-4-methylcoumarin (f.c. 416 µM, Bachem) and CaCl2 (f.c. 16.7 mM). Fluorescence was measured with a Fluoroscan Ascent reader (Thermo Labsystems) and data were analyzed with dedicated software (Thrombinoscope, Stago) [20]. Thrombin generation was expressed based on endogenous thrombin potential (ETP); lagtime (LT); thrombin peak (TP), time-to-thrombin peak (TTP).

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