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Regular Article

Effect of Pre-Analytical Conditions on the Thrombodynamics Assay

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ABSTRACT

Introduction: Standardization of pre-analytical conditions is the obligatory step for all potential diagnostic tests. Spatial clot growth (Thrombodynamics) is a new global hemostasis assay that considers spatial organization of coagulation. The principal parameter is rate of fibrin clot growth from the tissue-factor coated surface. In this work we studied the pre-analytical variables of Thrombodynamics assay that include conditions of blood collection, sample preparation and storage.

Materials and Methods: Blood of apparently healthy volunteers was used. Eight types of citrate blood collection tubes were tested, centrifugation conditions for plasma preparation were evaluated and impact of plasma freezing/thawing was tested.

Results: Among the blood collection tubes tested, BD Vacutainer glass tubes showed a significantly higher clot growth rate compared to plastic tubes. There was no difference between 3.2% and 3.8% of sodium citrate. For plasma preparation, a single 15 min centrifugation at 1 600 g shows significantly increased clot growth rate compared to plasma obtained by two sequential centrifugations (15 min 1 600 g, 5 min 10 000 g). There was no significant difference between 1 600 g and 2 100 g if the second centrifugation was performed. For the second centrifugation there was no difference between 20 min at 1 600 g and 5 min at 10 000 g. Frozen-thawed plasma showed increased clot growth rate compared to fresh plasma.

Conclusion: The data represent the necessary steps for the standardization of Thrombodynamics assay and for the formulation of the operating guide.

Introduction

During last years a new global hemostasis assay (Thrombodynamics) has been developed that is based on monitoring spatial clot formation initiated by immobilized tissue factor [1,2]. This assay considers not only biochemical reactions of the coagulation cascade but also the spatial organization of clot formation as the activator is not mixed with the plasma sample [3].

Coagulation in a thin layer of non-stirred plasma is initiated by immobilized TF and the fibrin clot growth into the bulk of plasma is monitored with a videomicroscopy system [1,4–6].

Spatial clot growth parameters were previously shown to be sensitive to hemophilia A, B and C [4–6] and coagulation state in sepsis [1]. It was also sensitive to the changes in the coagulation system due to platelet microparticles [7], recombinant activated factor VII [2,8] and to tissue factor pathway inhibitor inhibition [2].

To be used as a diagnostic method for coagulation disorders this method requires a validated standardization of the operating procedure. In this study we analyzed the effects of pre-analytical conditions (blood collection, plasma preparation and storage) on the results of spatial clot growth as a necessary step of this standardization.

Materials and methods

Blood donors

55 apparently healthy informed volunteers in total (29 males and 26 females; age range 22–65 years) who were recruited from the laboratory staff participated in this study.

Abbreviations: TF, tissue factor; APTT, activated partial thromboplastin time; PFP, platelet free plasma; PPP, platelet poor plasma.

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Plasma preparation

Unless otherwise stated, blood was collected into plastic tubes with sodium citrate at a 9:1 volume ratio using conventional straight Vacuette 21G needle, 0.8 × 38 mm (Greiner Bio-One, Kremsmunster, Austria). The first tube collected after the venipuncture was discarded. The blood was processed according to the standard protocol at our laboratory: the first centrifugation at 1 600 g during 15 min to obtain platelet-poor plasma (PPP) was followed by the second centrifugation 5 min at 10 000 g to obtain platelet-free plasma (PFP).

Blood collection tubes comparison

Commercially available citrated blood collection tubes from four manufacturers were used: Monovette plastic 4.5 ml 3.2% citrate (Sarstedt, Nümbrecht, Germany), Vacutainer glass 4.5 ml 3.2% citrate and plastic 2.7 ml 3.2% citrate (Becton Dickinson, Plymouth, UK), Vacuette plastic 4.5 ml 3.2%, 3.8% citrate and CTAD (Greiner Bio-One, Kremsmunster, Austria) and Venosafe plastic 4.5 ml 3.2% and 3.8% citrate (Terumo Europe N.V., Leuven, Belgium). The information about the tubes is summarized in Table 1.

Blood was collected from healthy volunteers in a random order into 4–6 different tubes. The first tube collected after the venipuncture was discarded. Sample preparation was performed as described in *Plasma preparation* section. Paired comparison was performed only for the tubes collected from the same volunteer.

Comparison of centrifugation protocols

All centrifugations were performed at room temperature. To compare the parameters of the first centrifugation two Monovette tubes (Sarstedt, Nümbrecht, Germany) were taken from each donor (n = 8). Immediately after blood collection the tubes were centrifuged 15 min at 1600 g or 2100 g. The plasma supernatant from the tubes was then centrifuged 5 min at 10 000 g. Twice-centrifuged plasma was used for the experiment.

To compare the parameters of the second centrifugation blood was collected into 5 ml Vacuette tubes (Greiner Bio-One, Kremsmunster, Austria) and centrifuged for 15 min at 1600 g. The supernatant was divided into two parts and centrifuged for 5 min at 10 000 g or for 15 min at 1600 g. The resulting PFP was treated as described above.

Plasma freezing

Blood was collected into 5 ml Monovette tubes (Sarstedt, Nümbrecht, Germany) and fresh PFP was obtained using two centrifugations: 15 min 2100 g followed by 5 min 10 000 g at room temperature.

300 μl of PFP were frozen at −80 °C and then thawed in water bath at 37 °C. The plasma samples were incubated at room temperature for 1 hour. Afterwards the samples were handled as fresh ones.

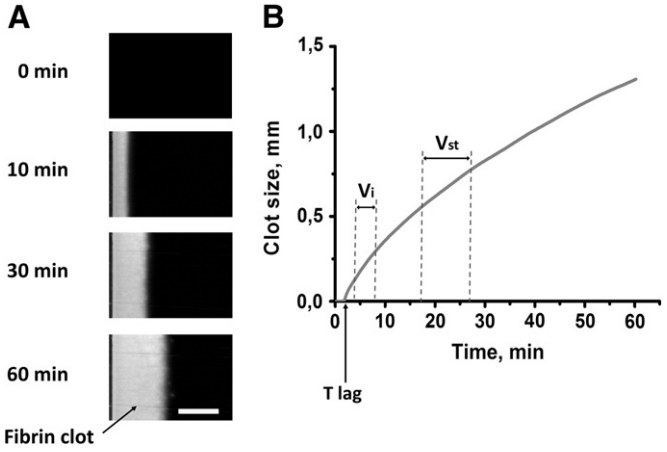

Fig. 1. Design of the Thrombodynamics assay. A. Typical images of growing fibrin clot. Coagulation is activated by immobilized TF (on the left) and fibrin clot grows into the bulk of plasma. Scale bar is 1 mm. B. Clot size vs. time plot calculated from the images. Lag time (T lag) is calculated as time between the moment of activation and actual start of clot growth. Rates of clot growth are calculated as a slope of the curve within the interval 2–6 min of clot growth (initial rate, Vi) and 15–25 min after beginning of clot formation (stationary rate, Vst).

Thrombodynamics assay

The general idea of the test was previously described in [1–3,6]. The scheme of the assay is shown on Fig. 1. Briefly, coagulation is activated in a thin layer of plasma when it is brought in contact with TF immobilized on a plastic surface. Clot formation starts on the activator and propagates into the bulk of plasma where there is no TF present.

Light scattering by fibrin allows observation of spatial clot formation in a real time by using a time lapse imaging (Fig. 1*A*).

The main parameters of clot growth in space are lag time, initial and stationary rates of clot growth. Lag time (Tlag) is defined as time between clotting initiation and actual appearance of the fibrin clot (Fig. 1*B*). This parameter is mostly dependent on tissue factor density on the activating surface [9,10] and on the factors of the TF pathway. Initial rate of clot growth (Vi) is measured as a slope of the curve on a clot size vs. time graph during 2–6 minutes of clot growth when coagulation occurs in the region where diffusion of the active factors from the activator play the major role. The most peculiar parameter is stationary rate of clot growth (Vst) measured as a slope of the curve on a clot size vs. time graph within the interval 15–25 min after clot growth beginning. Coagulation process occurs far from the activating surface without direct contact with TF and it is determined only by plasma protein properties. Factors of intrinsic pathway were shown to be responsible for self-sustained coagulation propagation in this system [3,5,6].

Thrombodynamics Analyser and Thrombodynamics kit (Hemacore LLC, Moscow, Russia) that includes the necessary reagents for the sample preparation and coagulation activation were used for all experiments [1]. For clotting activation TF immobilized to the plastic surface was used [9].

Table 1
List of the blood collection tubes used.

Tube name	Anticoagulant	Designation	Wall material
BD *Vacutainer*	105 mM (3.2%) of sodium citrate	Vacutainer glass	Siliconized glass
	109 mM (3.2%) of sodium citrate	Vacutainer plastic	Plastic
Sarstedt *Monovette*	106 mM (3.2%) of sodium citrate	Monovette	Plastic
Greiner Bio-One *Vacuette*	3.2% of sodium citrate	Vacuette 3.2%	Plastic
	3.8% of sodium citrate	Vacuette 3.8%	Plastic
	CTAD, 110 mM sodium citrate (3.2%) with theophylline, adenosine and dipyridamole,	Vacuette CTAD	Plastic
Terumo Europe *Venosafe*	109 mM (3.2%) of sodium citrate	Venosafe 3.2%	Plastic
	129 mM (3.8%) of sodium citrate	Venosafe 3.8%	Plastic