Anticoagulation Monitoring in Cardiovascular Disease

Introduction
Basic principles of anticoagulation testing
Specific laboratory tests
Prothrombin time
Whole blood Clotting times (a PTT and ACT)
Dry Reagent Technology (Heparin management test)
Ecarin Clotting Test
Viscoelastic Measures of Coagulation (Thromboelastography & Sonoclot Analyzer)
ENOX test
Platelet Monitoring tests

Introduction

Inhibition of normal hemostatic mechanisms is the cornerstone of medical therapy for many cardiovascular diseases, especially acute coronary syndromes and percutaneous coronary interventions. However if antithrombotic therapy is to be of value, a delicate balance has to be maintained between prevention of pathologic thrombosis and and excess risk of significant hemorrhage. Thus therapeutic administration of these agents must be guided by appropriate clinical and laboratory tests to assess risk of bleeding or continued thrombosis. Multiple types of assessments are possible, including individual enzyme inhibition[1-3], quantitation of thrombosis byproducts[4,5], and overall measures of clotting efficiency[6-8]. In this chapter discusses the physiological and clinical basis of the more commonly used laboratory tests for monitoring anticoagulation in patients with cardiovascular diseases. Some of the newer and promising tests will also be discussed.

Basic principles

Beneath the apparent simplicity of the routine coagulation times lies the incredible complex interplay of enzymes, coenzymes and inhibitors that combine to produce coagulation (figure1). Blood and plasma coagulation tests are a laboratory reproduction of events resulting in fibrin polymer formation, with or without platelet assistance. However the two processes are not necessarily synonymous. Coagulation tests are inititiated artificially, within the static confines of a test tube, often without the presence of erythrocytes or platelets. In-vivo thrombosis is far more complex and may not be adequately represented (or in some cases be misrepresented) by in-vitro tests[9]. For instance. The presence of lupus anticoagulant, while prolonging in vitro coagulation, confers an increased risk of thrombosis[9].The two principles that govern laboratory measures of coagulation are: the need for activation of an enzyme pathway and the need for a functional cofactor to assist enzyme function[10-12]. The characteristics of an

individual test are determined by the activator and whether or not there is excess of the necessary cofactors, such as phospholipids membrane. With the activator supplied in a simplified test tube reproduction, which obviates many of the regulatory and counter regulatory mechanisms, the primary limitation to thrombus initiation and growth is cofactor availability.
The endpoint of a test is the time at which sufficient fibrin has been generated to be detectable. Fibrin polymerization may be detected by a variety of methods including, the appearance of a visible fibrin clot, an increase in the optical density of the specimen, or a change in the mechanical resistance (using magnetic field manipulation or electrical conduction; figure 2[13].

Specific Laboratory Tests to monitor anticoagulation

Prothrombin Time

When tissue factor and a suitable phospholipid membrane are added to recalcified, platelet-poor plasma, the extrinsic pathway is activated, producing thrombin and fibrin clot[11,14,15]. Devised in the 1930's by AJ Quick[16], the prothrombin time (PT) test is based on the fact that in the presence of excess tissue factor, the efficiency of fibrin formation depends on the presence and activity of factors VII, X and V, and of thrombin and fibrinogen. The test is usually performed in a glass tube with an inner coating to prevent the activation of factor XII. Briefly, citrated platelet poor plasma is added to a calcium and thromboplastin suspension and the timer is started. The end of the test is marked by detection of fibrin polymerization, usually detected by change in the optical density of the specimen. The ratio of clotting time of test plasma to control plasma gives an estimate of the function of the extrinsic pathway clotting factors. The amount of tissue factor supplied during the test makes the activity of factor VIIa, rather than the tissue factor, the primary determinant of extrinsic pathway activation[17]. Therefore, PT, which hinges on factor VII function can be used to estimate the severity of factor production abnormalities, such as liver dysfunction, warfarin effect or vitamin K deficiency. Of note, Heparin and the specific antithrombins, also prolong PT[18,19]. However the plasma activity of heparin required to prolong PT is close to 1.0 U/ml. Because most commonly used heparin regimens attempt to achieve heparin concentrations near 0.4U/ml, this is not of much clinical utility.

There may be substantial interlaboratory variation in PT of identical specimens due to the differences in the commercially available thromboplastin preparations[20-22]. These preparations differ in their capability to initiate and support coagulation factor activity due to many factors, including the amount and activity of tissue factor supplied and the physical properties of the accompanying phospholipid membrane[23]. A particularly powerful tissue factor-phospholipid preparation may overcome a mild deficiency in factor activity, producing a normal PT, whwereas a weaker preparation may produce a prolonged PT and differences in the ratio of test to control PT. Although long suspected, the effect of these different thromboplastins was clearly established in a multinational study of the treatment of venous thrombosis, which reported an increased risk of bleeding when drug dosing was guided by prothrombin time ratios in North America[24]. A method

of controlling variations in thromboplastin sensitivity to the effect of warfarin was established in 1983[23,25]. This standardization method employs the International Normalized Ratio (INR), which is a mathematical computation of the ratio of the activity of the thromboplastin to be used to a reference thromboplastin held by the World Health Organization[25]. When prothrombin times using the different reagents are plotted on a logarithmic scale, there is a linear relationship between the different values. The slope of this relationship is a function of an individual thromboplastin's sensitivity to the effects of factor depletion and is known as the International Sensitivity Index (ISI). Reference thromboplastins have an ISI value of 1.0. Less sensitive preparations have a value near 2.0. Using ISI, an INR can be calculated according to the following formula: INR = (PT ratio)^{ISI} .[25,26]

Whole Blood Clotting Time

LEE and White described the whole blood clotting time (WBCT) in 1913 as a method for evaluating the intrinsic capability of blood to clot[27]. WBCT is based on the principle that when blood is placed in a glass tube, the intrinsic pathway to thrombin generation is activated and the initial thrombin generation is limited by the availability of cofactors that augment enzyme function, especially factors VIIa and Va, and calcium and a phospholipids membrane[10,14,28,29]. The initial appearance of active thrombin, albeit in low concentrations, leads to the generation of necessary cofactors through its action on factors VIII, V and platelet activation[10,14,28,30,31]. A burst of thrombin generation and fibrin polymerization ensues. This test does not utilize exogenous activators, except the glass tube.

The WBCT is primarily of historical interest now because the test methodology and results varied greatly. However, among its many derivatives, the *activated partial thromboplastin time (aPTT)* and the *activated clotting time (ACT)* are in widespread contemporary use.

Activated Partial Thromboplastin Time

In 1950s, a group of investigators developed a variation of the PT that used a lipophilic extract of mammalian (non human) brain for thromboplastin preparation[32], they used platelet poor plasma and an excess amount of a membrane substitute. They called this test PTT as they thought that their thromboplastin must be incomplete, as unlike the PT, it gave abnormal results with plasma from hemophiliacs[33]. The aPTT is a modification of the above, which avoids variation in the intensity of activation by initiating activation (during a pretest incubation period) with a constant quantity of activator (kaolin, celite etc.). When citrated, platelet-poor plasma is exposed to strong contact activation, is recalcified and platelet membrane substitute is supplied, the time required for fibrin clot to form is dependent on the integrity of the intrinsic pathway. The aPTT avoids the extrinsic pathway by excluding tissue factor from its reagents and also separates coagulation from platelet function.

The normal range for aPTT for most laboratories is 20-40 seconds (sec). It is prolonged when any of the factors from contact activation to fibrin polymerization are deficient or are inhibited to les than 30% of normal activity[34-36].

When heparin is complexed with antithrombin III (AT III), the ability of the heparin-AT III to inhibit thrombin may be increased 1000 fold over that of AT III alone[37]. As a result, the initial steps following contact activation proceed slowly through the regulatory points. The small amount of thrombin produced decays at an accelerated rate, and the activation of factor VIII is impaired. Consequently more time is required to produce thrombin in sufficient concentrations to effectively overcome the rate limiting steps in the intrinsic pathway (Figure 36.4 old chapter). This lag time is the measurable prolongation of clotting times produced by the thrombin inhibitors (such as heparin; figure 36.5 –old chapter)[10,12,38]. Because aPTT has a strong contact activation step, it is intermediately sensitive to the presence of thrombin inhibitors. The aPTT is prolonged at a level of thrombin inhibition that correlates with heparin activity of 0.1-0.6 U/ml, a range known to be clinically effective for medical treatment of venous and some arterial thrombosis[6,39-41]. The aPTT is the most widely used test for guiding heparin therapy for venous and arterial thrombosis and for medical management of acute coronary syndromes (ACS), with usual target range of 1.5-2.5 times the control value[6,37,39,40]. However, when heparin is administered to achieve higher concentrations (as in the setting of percutaneous intervention [PCI] or cardiopulmonary bypass)[42-44], the aPTT is nonlinear in its response to heparin and may be almost infinitely prolonged; the aPTT in these circumstances is no longer a useful test[7,45-47].

Direct thrombin inhibitors (DTI), in contrast to heparin are active against fibrin-bound thrombin and like heparin, not only do they inhibit thrombin production and activity, they may also inhibit thrombin induced platelet activation[48,49]. Thus, because DTI have different mechanisms of action and different dose-effect relationships, the relationship between aPTT prolongation and antithrombotic effect will be different from that of heparin. A given aPTT range for DTI may correspond to an equal or greater antithrombotic effect than for heparin, especially in situations where the thrombosis is platelet dependent(as in ACS)[50,51]. Thus direct comparisons of drug efficacy cannot necessarily be made based on equal prolongation of aPTT[51]. Risk/benefit ratios, with respect to measures of antithrombotic effect, must be determined separately for anticoagulants of different classes. Therefore, antithrombotic recommendations using the aPTT, must not only specify the reagents but also the type of antithrombotic therapy used.

Point of care testing

Conventionally, the aPTT is performed in A central laboratory. This involves a substantial delay in the reporting of results from the time of ordering the test[52]. A survey of 79 hospitals that participated in the GUSTO 1 trial showed that the mean time from blood draw to availability of the aPTT result was 1 hour 46min[53]. Since patients with acute coronary syndromes would benefit from a more rapid turnover tome for aPTT,

several point of care anticoagulation systems are now commercially available and have clinically been tested. There are three commercially available bedside aPTT monitors in the united states designed for use in critical care settings. Each provides a rapid determination of the aPTT (typically within 3 minutes). Table 1 provides a comparison of these point of care devices.

Activated Clotting Time

ACT, first described by Hattersley in 1966,[54] is essentially a modified WBCT in which an attempt has been made to standardize contact activation by using a constant amount of kaolin or celite. The ACT is similar to the aPTT in its dependence on the contact activation pathway for thrombin generation, but the ACT does not supply an excess of phopholipid membrane. In the aPTT, the phopholipid area is supplied in excess so that it will not interfere with the of coagulation enzyme function. The ACT requires that this area be developed through platelet activation during the testing process. This induces an additional variable to the function of coagulation enzymes. In the ACT following contact activation, the first thrombin to appear must activate platelets, in addition to factors V and VIII, and any alteration in endogenous platelet function may also alter ACT. Thus, rather than being a pure test of coagulation enzyme function, the ACT is also a somewhat imprecise measure of the cooperation between the intrinsic pathway and platelet function. For this reason (as will be detailed in a later section), ACT is useful in monitoring the combined use of heparin and glycoprotein IIb/IIIa. Also, the ACT is less sensitive to low levels of heparin anticoagulation than aPTT. However it maintains a good correlation with the heparin effect at higher heparin doses[7,55,56] and thus is widely used for guiding heparin therapy in clinical situations needing high dose heparin such as cardiopulmonary bypass and PCI[42-46].

Unlike, Hattersley's original assay[54], that required manual mixing of a blood sample with the contact activator and visual assessment of the tube for time to visible clot formation, commercial automated ACT monitors now in routine use. The Hemochron system (International Technidyne, Edison, NJ) uses a celite activator and detects fibrin polymerization using an oscillating magnetic field. The HemoTec (Medtronic HemoTec, Englewood, CO) uses a kaolin activator and measures the rate of plunger fall to detect fibrin polymerization. Thus, system activators and fibrin detection mechanisms differ, giving different sensitivities to factor depletion and inhibitor therapy. Although there is reasonable correlation, the values of one system cannot be extrapolated to the other systems[57]. Thus, any recommendations for anticoagulation intensity, measured by ACT, must be qualified by the specific type of device used[58].

The normal ACT range in general is 90-130seconds. Due to the range of variation in normal values, effects of low dose anticoagulants may go unnoticed in individual patients. The ACT may be shortened during ongoing thrombosis, such as in surgical procedures or unstable coronary syndromes, largely as a function of the availability of activated platelets. Contamination of blood samples with activated platelets (as with indwelling catheters) may also shorten ACT. Also, aprotinin inhibits contact activation by celite (diatomaceous earth)[59]. If this interaction is not considered, then an insufficient

dose of heparin may be administered in this setting. Thus kaolin ACT should be used in patients receiving aprotinin.

Because the aPTT and ACT measure similar phenomenon, (except for the contribution of activated platelets), there is a fair correlation between the tests when assessing heparin therapy[45]. However, DTI, in addition to impairing the feedback amplification of coagulation enzyme function, also prevent thrombin induced platelet activation[48,49]. The resultant denial of factors VIIIa and Va and a phospholipids surface prolongs ACT. In drug concentration ranges at which the two tests are responsive, antithrombins produce a proportionately greater rise in ACT than aPTT[51]. Thus, at drug concentrations producing an equivalent rise in the aPTT, the ACT increase with specific thrombin inhibitors is significantly greater than that with heparin. Powerful antiplatelet drugs like glycoprotein IIb/IIIa inhibitors, may prolong ACT[60]. Thus ACT may be a good tool to assess the intensity of antithrombotic drugs when combined anticoagulants and antiplatelet agents are used in a clinical syndrome that may involve endogenous platelet activation (as in PCI setting).

More recently, another point of care ACT monitoring device has become available. The i-STAT system (i-STAT Corp., Princeton) is modular and consists of a handheld unit into which disposable cartridges are placed. The cartridges are self contained and they need <1ml of blood. There are individual cartridges available to measure electrolytes, blood gases and level of anticoagulation (ACT)[61,62]. Each i-STAT unit can download patient data to a central computer for storage and subsequent retrieval. Unlike traditional ACT, the i-STAT ACT is not based on the formation a stable thrombus. Instead a substrate marker releases an electric signal when it is cleaved by active thrombin. The time to generation of the electroactive marker is reported as the ACT[63]. Therefore, the i-STAT is less susceptible to fibrinogen levels, temperature, hematocrit, dilution and the addition of IIb/IIIa inhibitors. Because the production of thrombin occurs earlier than the formation of a stable clot, one would expect the value of the i-STAT to be lower than that of the Hemochron ACT. In this regard, Schusssler et al compared the i-STAT ACT with Hemochron ACT during and after PCI[63]. They found a high degree of correlation between the two ACTs both at low and high level of anticoagulation. Statistically, there was no difference between these two devices at lower levels of anticoagulation; at higher levels of anticoagulation there was a statistically significant difference that was not thought to be clinically significant. The use of IIb/IIIA inhibitors did not significantly change these relationships. Due to small numbers of patients, no conclusion could be drawn for use with DTI.

Dry Reagent technology

Rather than using separate solutions that must be added to a test tube, the dry reagent technology, places the coagulation activator and cofactors on a card. A very small amount a citrated blood is placed in a reaction chamber; the particular reagent preparation used determines the type of clotting time that is performed. Thus, if the same detection

system is used, a single analyzer may be used for a variety of different tests. The Heparin Management Test (HMT) (Cardiovascular Diagnostics, INC., Raleigh, N.C.), based on this technology, is a measure of whole blood anticoagulation and was introduced as an alternative to conventional ACT measurements. It is similar to ACT in principal (uses celite as activator), but uses a microprocessor-controlled analyzer and disposable test cards. A reaction chamber within each test card contains paramagnetic iron oxide beads and dry chemical reagents necessary to activate the coagulation cascade in the blood sample[64]. After a drop of blood is added, capillary action draws a small portion of of this blood into the reaction chamber. An oscillating magnetic field is applied to the blood, chemical reactants and to the beads. An infrared light beam passed through the test chamber detects oscillations in the amplitude of transmitted light coincident with the bead movement in the test chamber. As clot formation occurs, the beads become enmeshed within the clot, reducing the amplitude of light oscillations to trigger the end time for the HMT measurement Figure 3. The system come as an analyzer (TAS/ Rapidpoint Coag analyzer®) and a variety of test cards that can be used to monitor different aspects of anticoagulation. The low HMT cards are used to monitor low to moderate levels of unfractionated heparin (heparin concentration from 0.25 to 3.0 U/ml) such as during PCI while the HMT test cards are intended to monitor the effects of higher doses of heparin (1.0-10.0 U/ml). The test system can use both citrated and non citrated whole blood. Table 2 compares the salient features of some of the available whole blood coagulation analyzers. The TAS HMT has been compared to the Hemochron and HemoTec ACT in prospective studies in the PCI setting. Tsimikas et al noted that the the TAS-HMT gave significantly higher readings (about 15% higher) than the HemoTec ACT but a good correlation was noted between the two methods (r = 0.77), and the relation was similar in patients who received IIb/IIIa inhibitors[65]. However, at ACT values greater than 300seconds the correlation was less strong. When compared to the Hemochron ACT, the two systems gave fairly similar values (HMT292 ± 33 sec and ACT 284 ± 31 sec) and there was a reasonable correlation (0.66)[66,67]. Also HMT was shown to have a good correlation with anti-factor Xa activity in patients undergoing cardiovascular surgery[68].

Ecarin Clotting Time

In most large clinical trials aPTT has been used to monitor heparins; it is also the most commonly used assay to monitor heparin in clinical practice. Also, the manufacturers of most direct thrombin inhibitors (DTIs) recommend aPTT to these agents[69-71]. However, unlike heparins that indirectly inhibit factors IIa, Xa, IXa, XIa and XIIa, DTIs only inhibit thrombin[72]. On the other hand, aPTT reflects inhibition of factors IIa, IXa and Xa[73]. Thus intuitively, aPTT would not be the optimal method of monitoring DTIs because its values are affected by clotting factors not directly influenced by DTIs[74]. Data in human studies has showed only a moderate correlation of the concentration of these agents to aPTT values[75,76].The ecarin clotting time (ECT), on the other hand is used specifically to monitor the effect of DTIs. Ecarin, used as a thrombin generating agent in the ECT, is derived from the venom of a snake (Echis carinatus). Ecarin cleaves the

arginine 320-isoleucine 321 protein bond of prothrombin, thereby generating meizothrombin. Meizothrombin possesses thrombin-like proteolytic activity. Like thrombin, its active site is inhibited by direct thrombin inhibitors (DTI). The principle behind the ECT is that after the addition of a specific quantity of ecarin to blood containing a DTI, meizothrombin is generated. Meizothrombin then reacts with the DTI to neutralize it. Once the inhibitor is neutralized, the remaining free Meizothrombin can activate the clotting process by stimulating the conversion of fibrinogen to fibrin. Since, heparins are poor inhibitors of meizothrombin, the ECT is relatively specific for DTIs[77,78].

Clinical use of ECT has been reported. One study examined the precision of ECT compare to aPTT for monitoring lepirudin in 10 patients with history of heparin induced thrombocytopenia, undergoing open heart surgery[79]. These investigators found a strong linear relationship between the ECT values and lepirudin concentrations that ranged from 0.35-5.88μg/ml (r=0.94), whereas the aPTT demonstrated only a weak correlation (r=0.61). Other studies also have shown that ECT correlates better with lepirudin levels and demonstrates less interpatient variability compared with the aPTT[80] or the ACT[78,80].

The thrombin inhibitor management test (TIM) (Pharmanetics, Morrisville, NC), a point of care test, based on the ECT has been developed using the dry reagent technology similar to that in the HMT test cards[81]. Cho et al compared the TIM ECT test and ACT [using the Hemochron ACT (International Technidyne, Edison, NJ) and Coagucheck Pro/DM (Roche Diagnostics, Indianapolis, Indiana) with central laboratory anti-factor IIa assay for monitoring bivalirudin-mediated anticoagulation in the setting of non-emergency PCI[82]. All 64 patients received bivalirudin bolus of 0.75mg/kg followed by a 1.75mg/kg/hr infusion. 55 patients also received concomitant IIb/IIIa inhibitors. Samples were drawn at baseline, after administration of bivalirudin, after IIb/IIIa inhibitor bolus and during the procedure and at sheath pull. The results showed that TIM ACT provided more accurate assessment of bivalirudin during PCI than ACT Figure 4. After IIb/IIIa inhibitor administration, no significant change in bivalirudin concentration or ECT values was noted, though the Hemochron ACT values increased. Thus, serum DTI concentrations correlate with the degree of clotting time prolongation and the ECT may be an alternative to monitoring patients' DTI levels[83].

Viscoelastic Measures of Coagulation

Initially developed in the 1940s, viscoelastic measures of coagulation have undergone a resurgence in popularity. These tests are unique as they can measure the entire spectrum of clot formation from early fibrin strand generation, through clot retraction and eventual fibrinolysis. Currently two such devices are in clinical use and will be described.

Thromboelastography

Thromboelastography was first described in 1948 by Hartert[84]. With the advent of computerization, this has evolved from a research tool into a compact, commercially available point of care instrument : The Thrombolestagraph (TEG) (Hemoscope;Morton Grove, Ill).

It consists of a heated (37°C) cuvette that holds the blood (0.36ml) as it oscillates through an angle of 4°45´. Each rotation lasts 10 seconds, which includes a 1 second rest period at the end of excursion. A pin, which is suspended freely in the blood by a torsion wire, is monitored for motion (Figure 5). The torque of the rotating cup is transmitted to the pin once the clot starts to form. Therefore, the strength and rate of these fibrin-platelet bonds affect the magnitude of pin motion. When the clot lyses, the bonds are broken and the transfer of cup motion is diminished. The rotation of the pin is coverted by a mechanical-electrical transducer to an electrical signal that can be monitored and recorded by a computer. Thus, TEG documents initial fibrin formation, clot rate strengthening, and fibrin platelet bonding via GP IIb/IIIA to eventual clot lysis (Figure 6).

The strength of a clot is graphically represented over time as a characteristic cigar shaped figure. There are 5 parameters of the TEG tracings which measure different stages of clot development[85-87] (Figure 6):

R: The **R** value or the reaction time is a period of time from initiation of the test to the initial fibrin/clot formation (normal: 7.5-15min). It is considered comparable to the whole blood clotting time and may be accelerated by adding celite to the sample cuvette. The **R** value is prolonged by a adeficiency of one or more plasma coagulation factors and shortened in hypercoaguable states.

K: This is measured from **R** time (i.e. from the beginning of clot formation) until the level of clot firmness reaches 20mm (divergence of the lines from 2-20mm). Therefore, **K** is a measure of clot strengthening. It is shortened by an increased fibrinogen level and, to a lesser extent , by increased platelet function and is prolonged by anticoagulants.

α: Alpha angle is formed by the slope of the TEG tracing at **R** from the horizontal line. It represents the acceleration kinetics of fibrin build up and cross linking. Like **K** it is also increased by increased fibrinogen levels and, to a lesser extent , by increased platelet function and is decreased by anticoagulants. In hypercoaguable states, in which the clot amplitude never reaches 20mm (i.e. K is undefined), the angle is more comprehensive than **K** time.

MA: Maximum amplitude (MA) reflects strength of a clot which is dependent on the number and function of platelets and its interaction with fibrin. It may be

decreased by either qualitative or quantitative platelet dysfunction or decreased fibrinogen concentration. Normal MA is 50-60mm.

CI : The coagulation index describes the patients overall coagulation status. It is derived from the **R**, **K**, **MA** and **α** of native or celite activated whole blood tracings (**CI** for celite activated blood = 0.3258**R** – 0.1886**K** + 0.1224 **MA** + 0.0759 **α** – 7.7922). Normal values range from -3.0 to +3.0, which is equivalent to 3 standard deviations about the mean of zero. Positive values outside the range (**CI**>3) indicate that the sample is hypercoaguable, whereas negative values outside this range (**CI**<3) indicate that the sample is hypocoaguable.

Ly30/LY60: These values measure percentage lysis at 30 and 60 minutes, respectively, after the **MA** is reached. Measurmenst are based on the reading of the area under the TEG tracing from the time **MA** is measured until 30 and 60 minutes after the **MA**. Therefore, when these values are high, the fibrinolytic activity is high.

TEG tracings can be qualitatively and quantitatively analyzed[88]. Various patterns can be easily recognized (Fig7) as hypocoagulation, normal coagulation, hypercoagulation and fibrinolysis. However by using measurements and established normal ranges and indices, the patterns can be quantified as to the degree of abnormality, which allows better monitoring of therapies. Measurements derived from these diagrams have been related to more traditional measures of coagulation, such as ACT[89]. TEG monitoring has been used in liver transplant surgery to rapidly analyze and treat the changing coagulation profile of the patients[90]. Studies have evaluated the utility of TEG in cardiac surgery after cardiopulmonary bypass (CPB)[91] and to assess blood product transfusion requirements. Shore-Lesserson et al[91] compared transfusion requirements in arandomized, prospective trial of high risk cardiac surgical patients. Patients were randomly assigned to TEG guided transfusion therapy or standard laboratory based transfusion therapy. Patients in both group received antifibrinolytic therapy with EACA. They noted that patients in the TEG group received fewer total transfusions and a significantly less volume of fresh frozen plasma. The authors concluded that TEG based transfusion algorithm reduced transfusion requirements. The TEG may also be used to differentiate surgical bleeding from coagulopathy following cardiac surgery[92]. The TEG has also been used successfully to monitor hirudin therapy during CPB in the setting of heparin induced thrombocytopenia[87]. Some of the other clinical applications of TEG have been to assess the coagulation status in obstetrical patients receiving low molecular weight heparins[93] in the peripartum period and in disseminated intravascular coagulation[94,95].

Sonoclot Analyzer

The Sonoclot® (Sienco, Inc; Morrison, Colo) provides an alternative viscoelastic measure of coagulation. Compared with TEG, the sonoclot immerses a rapidly vibrating probe into a 0.4ml sample of blood. As the clot formation occurs, impedance to probe

movement through the blood increases and generates an electrical and a characteristic 'clot signature'. The sonoclot may be used to derive the ACT as well as provide information regarding clot strength and clot lysis[96]. The Sonoclot generates both a qualitative graph, known as the Sonoclot signature (figure8) and quantitative results on the clot formation time (ACT-onset) and the rate of fibrin polymerization (Clot rate) for identifying numerous coagulopathies including platelet dysfunction, factor deficiencies, anticoagulation effect, hypercoaguable tendencies and fibrinolysis. As the blood sample clots, numerous mechanical changes related to the performance of the patients hemostasis system occur that alter the clot signal value[97]. The record of the clot evolution is saved as a graph of the clot signal versus time. Both celite and kaolin activated Sonoclot ACT tests are available[98].

In a Sonoclot signature, the coagulation cascade reactions develop from the beginning and continue throughout the liquid phase (represented by the initial horizontal portion of the graph in figure). This phase ends when the viscosity of the sample increases with thrombin generation and resulting intitial fibrin formation and represents the ACT (above figure). This followed by the continued conversion of fibrinogen to fibrin and its polymerization into a gel. This is affected by both the rate of conversion to fibrin and the availability of fibrinogen. This phase is represented by the slope of the graph (clot rate) and by the height of the signature when the gel formation is completed. This information is important in clinical applications including monitoring anticoagulants (figure from monograph showing heparin effect), hypercoaguable states and fibrin hemodilution. Sonoclot also responds to clot retraction occurring within the test sample. As the clot retracts it tightens causing the signature to rise. Eventually, the clot often pulls away from some of the surfaces of the cuvette or probe. This results in a fall in the graph.
The sonoclot has been used to monitor the coagulation status in a multitude of conditions including cardiac surgery[89], hemodialysis patients[99] and malignancy associated. hypercoaguable states[100].

ENOX Test

The ENOX test® (Pharmanetics Inc., Morrisville, NC) measures the combined anti-Xa and anti-IIa activity of the low molecular weight heparin (LMWH) enoxaparin[101]. Unlike heparin that has an anti-Xa/anti-IIa activity ratio of one, the LMWHs have a much higher relative anti-Xa inhibition. This is an important distinction as the ability to prolong the aPTT and ACT is proportional to the anti-IIa activity. The ENOX test was developed for exculsive use with the LMWH enoxaparin. In the ENOX test, factor X is rapidly converted to factor Xa by a specific activator of factor X, initiating the clotting process. Enoxaparin, from the patients blood, complexes with antithrombin to inhibit factor Xa and proportionately lengthen the clotting time. Conventional chromogenic anti-Xa assays provide drug concentrations only in dilute, supplement plasma and are not suitable for point of care use. The ENOX test is commercially available for point of care use and is based on dry reagent technology. The ENOX test cards are manufactured to be used with the Rapidpoint® Coag analyzer (Bayer Corp). As with all similar tests, all the components needed to perform the test, with the exception of the patient sample, are included in the

reaction chamber of the test card[101]. The test uses one drop (35µl) of citrated venous or arterial blood sample. The ENOX test was designed to measure citrated whole blood clotting times corresponding to enoxaparin concentration of 0.0-3.0 anti-Xa IU/ml in derived plasma[102]. In a clinical trial that included patients undergoing PCI using enoxaparin, the ENOX clotting times correlated well (r=0.80) with chromogenic anti-Xa assay derived plasma enoxparin concentrations (range <0.1 – 1.8IU/ml)[103]. Based on prior studies, a proposed targeted window for PCI anticoagulation with enoxaparin is 0.8-2.0 IU/ml[104-106]. This corresponds to an ENOX clotting time of 250-450 seconds[104-106]. The recently completed ELECT trial (Evaluation of ENOX clotting times) will shed further light on the applicability of ENOX test in PCI.

Monitoring of Platelet function

The pivotal role of the platelet in arterial thrombosis in general, and in acute coronary syndromes in particular, is well established. It is also clear that inhibition of platelets with an antiplatelet agent (such as aspirin, the thienopyridines, or the platelet glycoprotein IIb/IIIa inhibitors) can reduce progression to myocardial infarction in unstable coronary syndromes and reduce the incidence of ischemic complications during PCI. However despite the effectiveness of these agents, they fail to prevent thrombotic events in all patients. One of the explanations for this, is a potential heterogenous response among indivisuals to standard dosing regimens. Current clinical practice (and practice guidelines)[107] do not include any measurement of the effectiveness of platelet inhibitor therapy. Significant variation in the response to aspirin has been demonstrated by various studies[108,109].

However, only recently, after the introduction of glycoprotein IIb/IIIa inhibitors has there been an expansion in our capabilities to assess platelet inhibition[110]. Platelet function tests measure the capacity of platelets to adhere, activate, aggregate and secrete. The goal of platelet function testing is to provide information about the platelet contribution to the risk of thrombosis or hemostasis. Important clinical questions in acute coronary syndromes are whether the antiplatelet agent is having the desired effect on platelet inhibition (inhibition) whether the patient has sufficient platelet function to avoid significant bleeding (safety).

Platelet thrombus formation involves a number of processes, including activation of the platelet, secretion of vasoactive and prothrombotic chemicals, promotion of the clotting cascade, and platelet aggregation, each of these indivisual platelet functions may be affected by platelet therapy and could form the basis for a functional assay[110]. It is not clearwhich function is most important to influence therapy or monitor with testing. A variety of platelet function tests are available for use in the central laboratory, of which the ***Photo-Optical Tubidometric Aggregometric Assay*** is considered the gold standard. This test involves, addition of a platelet aggregation agonist (eg. ADP, collagen, epinephrine, or thrombin receptor activating peptide [TRAP]), to platelet rich plasma. As platelets aggregate, light transmission increases. Maximum transmittance is calibrated

using platelet poor plasma while minimum transmittance is determined with platelet rich plasma prior to addition of the agonist.

For rapid clinical use in the critical care/ cardiac catheterization laboratory setting, various point-of-care tests have been described, of which the following are more commonly used clinically in cardiovascular settings.

Rapid Platelet Function Assay

The Ultegra-RPFA (Accumetrics, San Diego, CA) is an automated, whole blood, cartridge-based optical aggregometer that utilizes fibrinogen-coated polystyrene beads. After addition of an agonist (iso-TRAP), these beads agglutinate in whole blood in proportion to the number of unblocked glycoprotein IIb/IIIa inhibitors receptors[111]. The light transmittance increases with progressive binding of the platelets to the beads, leading to their agglutination. Current use involves, a baseline measurement and repeat tests after drug administration. The results can be reported as a percentage of baseline aggregation or as an absolute rate of aggregation. One of the drawbacks of the test, as is the case with most currently available tests, is that it differs from in vivo conditions in that it is not conducted under flow conditions. The RPFA has been been validated versus turbidometric aggregometry[112]. At increasing concentrations of abciximab, the percentage inhibitition of the RPFA correlated well with the turbidometric assay (r^2=0.95). This study also showed an excellent correlation with the degree of glycoprotein IIb/IIIa inhibitors receptor occupancy (measured using radiolabelled abciximab) r^2=0.96.

Initial animal studies with monoclonal antibody 7E3 demonstrated that blockade of ≥ 80% of glycoprotein IIb/IIIa inhibitors receptors with suppression of platelet aggregation to ≤ 20% of baseline was necessary to prevent in vivo thrombosis[113]. This level of platelet inhibition was used as the basis for the early dosing studies for abciximab (c7E3 Fab, ReoPro, Centocor, Inc., Malvern, PA, USA)[114] and the importance of maintaining this level of platelet inhibition in the clinical setting was not tested directly. Numerous small studies have used RPFA to measure platelet inhibition after administration of glycoprotein IIb/IIIa inhibitors[115,116]. The multicenter GOLD trial correlated the degree of platelet inhibition (using the RPFA device) to the clinical outcomes, in five hundred patients undergoing PCI with adjunctive glycoprotein IIb/IIIa inhibitors use[117]. Major adverse cardiac events (MACEs) were prospectively monitored./ One quarter of all patients did not achieve ≥ 95% inhibition 10 minutes after the bolus and experienced a significantly higher incidence of MACEs (14.4% versus 6.4%, p=0.006). Patients whose platelet function was < 70% inhibited at 8 hours after the start of therapy had a MACE rate of 25% versus 8.1% for those ≥ 70% inhibited (p=0.009). By multivariate analysis, platelet function inhibition ≥ 95% at 10 minutes after start of therapy was associated with a significant decrease in the incidence of a MACE (OR 0.46, 95% CI 0.22 to 0.96, P=0.04). The authors concluded that there was substantial variability in the level of platelet function inhibition achieved with glycoprotein IIb/IIIa inhibitors among patients undergoing PCI. Also that the level of platelet function inhibition as measured by RPFA was an independent predictor for the risk for MACEs after PCI.

Platelet Function Analyzer (PFA-100)

The PFA-100 test (Dade Behring, Miami, FL) evaluates primary hemostsis through platelet-platelet interaction as whole blood flows under shear stress conditions through an aperture[118]. The instrument uses citrated whole blood which is drawn by means of a vacuum through a capillary tube producing high shear forces and then through a precisely defined aperture in a membrane that has been coated with either collagen and epinephrine or collagen and ADP. Thje platelets adhere and aggregate at the aperture until it is occluded, and the results are reported as the closure time (CT). The testing process takes about 10 minutes. The use of whole blood allows for the interaction of platelets with red blood cells and other whole blood components that may play a role in thrombus formation. Also the addition of shear stress intp platelet activation may make this test theoretically more physiologically sound[119]. The test is prolonged by platelet counts below 50,000 and hematocrit levels below 25%. The normal range for CT for healthy subjects is 59-120 seconds[120]. The maximal CT has been set at 300 seconds, which represents nonclosure of the aperture after 300 seconds. In a study of healthy volunteers treated with different concentrations of Tirofiban, there was found to be a good linear correlation of turbidometric aggregation with PFA with collagen-epinephrine membrane percent maximal CT prolongation (r^2=0.97), and a strong quadratic correlation of turbidometric aggregation and PFA with collagen-ADP membrane percent maximal CT prolongation (r^2=0.098).

Conclusions

A variety of laboratory and point of care methods are available to help guide antithrombotic therapy. Depending on the clinical situation at hand, the appropriate test varies. For example, there is substantial evidence and clinical experience supporting the use of the standard aPTT in guiding heparin therapy for the prevention of pulmonary thromboembolism in patients with deep venous thrombosis. In contrast ACT is used to assess heparenization for angioplasty or bypass surgery. For direct thrombin inhibitors the conventional methods like aPTT or ACT may not be optimal and newer tests such as the ECT are being investigated. Viscoelastic measures of coagulation are also being investigated in various clinical settings including during cardiopulmonary bypass. Point of care tests are becoming increasingly popular especially in the acute care setting. In this regard it is important to have stringent rules and guidelines to ensure proper quality measures and calibration to ensure reliability. This is especially true as these devices typically have higher coefficients of variability than standard laboratory tests[121]. Finally, it should be remembered that though two test may measure the same parameter (for example ACT), there numeric values may not be (and usually are not) identical. Thus

each coagulation test value should be preferably qualified by the type of device used to measure it.

References:

1. Hoppensteadt DA, Walenga JM, Fareed J. Validity of serine protease inhibition tests in the evaluation and monitoring of the effect of heparin and its fractions. *Semin Thromb Hemost*. 1985;11:112-20.
2. Holm HA, Abildgaard U, Larsen ML, Kalvenes S. Monitoring of heparin therapy: should heparin assays also reflect the patient's antithrombin concentration? *Thromb Res*. 1987;46:669-75.
3. van den Besselaar AM, Meeuwisse-Braun J, Bertina RM. Monitoring heparin therapy: relationships between the activated partial thromboplastin time and heparin assays based on ex-vivo heparin samples. *Thromb Haemost*. 1990;63:16-23.
4. Mannucci PM, Bottasso B, Tripodi A, Bianchi Bonomi A. Prothrombin fragment 1 + 2 and intensity of treatment with oral anticoagulants. *Thromb Haemost*. 1991;66:741.
5. Hoek JA, Nurmohamed MT, ten Cate JW, Buller HR, Knipscheer HC, Hamelynck KJ, Marti RK, Sturk A. Thrombin-antithrombin III complexes in the prediction of deep vein thrombosis following total hip replacement. *Thromb Haemost*. 1989;62:1050-2.
6. Basu D, Gallus A, Hirsh J, Cade J. A prospective study of the value of monitoring heparin treatment with the activated partial thromboplastin time. *N Engl J Med*. 1972;287:324-7.
7. Congdon JE, Kardinal CG, Wallin JD. Monitoring heparin therapy in hemodialysis. A report on the activated whole blood coagulation time tests. *Jama*. 1973;226:1529-33.
8. Bounameaux H, Marbet GA, Lammle B, Eichlisberger R, Duckert F. Monitoring of heparin treatment. Comparison of thrombin time, activated partial thromboplastin time, and plasma heparin concentration, and analysis of the behavior of antithrombin III. *Am J Clin Pathol*. 1980;74:68-73.
9. Alving B. *Lupus anticoagulants, anticardiolipin antibodies, and the antiphospholipid syndrome.* Boston: Blackwell Scientific; 1994.
10. Hemker HC, Kessels H. Feedback mechanisms in coagulation. *Haemostasis*. 1991;21:189-96.
11. Rapaport SI, Rao LV. Initiation and regulation of tissue factor-dependent blood coagulation. *Arterioscler Thromb*. 1992;12:1111-21.

12. Hemker HC BS. The mode of action of heparins in vitro and in vivo. In: Lane, ed. *Heparin and related polysaccharides*. New York: Plenum Press; 1992:221.
13. Alson JD PB. *Automated coagulation systems*. New Yoek: Van Nostrand Reinhold; 1995.
14. Mann KG, Krishnaswamy S, Lawson JH. Surface-dependent hemostasis. *Semin Hematol*. 1992;29:213-26.
15. Tracy PB, Eide LL, Mann KG. Human prothrombinase complex assembly and function on isolated peripheral blood cell populations. *J Biol Chem*. 1985;260:2119-24.
16. Quick AJ S-BM, Bancroft FW. A study of the coagulation defect in hemophilia and jaundice. *Am J Med Sci*. 1935;190:501.
17. Morrissey JH, Macik BG, Neuenschwander PF, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. *Blood*. 1993;81:734-44.
18. Zoldhelyi P, Webster MW, Fuster V, Grill DE, Gaspar D, Edwards SJ, Cabot CF, Chesebro JH. Recombinant hirudin in patients with chronic, stable coronary artery disease. Safety, half-life, and effect on coagulation parameters. *Circulation*. 1993;88:2015-22.
19. Sharma GV, Lapsley D, Vita JA, Sharma S, Coccio E, Adelman B, Loscalzo J. Usefulness and tolerability of hirulog, a direct thrombin-inhibitor, in unstable angina pectoris. *Am J Cardiol*. 1993;72:1357-60.
20. Hirsh J, Levine M. Confusion over the therapeutic range for monitoring oral anticoagulant therapy in North America. *Thromb Haemost*. 1988;59:129-32.
21. Eckman MH, Levine HJ, Pauker SG. Effect of laboratory variation in the prothrombin-time ratio on the results of oral anticoagulant therapy. *N Engl J Med*. 1993;329:696-702.
22. Bussey HI, Force RW, Bianco TM, Leonard AD. Reliance on prothrombin time ratios causes significant errors in anticoagulation therapy. *Arch Intern Med*. 1992;152:278-82.
23. Kirkwood T. Combination of refernce thromboplastins and satndardization of the prothrombin time ratio. *Thromb Haemost*. 1983;49:238.
24. Hull R, Hirsh J, Jay R, Carter C, England C, Gent M, Turpie AG, McLoughlin D, Dodd P, Thomas M, Raskob G, Ockelford P. Different intensities of oral anticoagulant therapy in the treatment of proximal-vein thrombosis. *N Engl J Med*. 1982;307:1676-81.
25. Thompson J. *The implementation of international normalized ratios for standardization of the prothrombin time in the oral anticoagulant control*. New York: Churchill Livingstone; 1991.
26. Poller L, Keown M, Chauhan N, Shiach C, Van Den Besselaar AM, Tripodi A, Jespersen J. European Concerted Action on Anticoagulation (ECAA): International Normalized Ratio Variability of CoaguChek and TAS Point-of-Care Testing Whole Blood Prothrombin Time Monitors. *Thromb Haemost*. 2002;88:992-5.
27. Lee RI Wp. A clinical study of coagulation time of blood. *Am J Med Sci*. 1913;145:495.

28. Jesty J. Interaction of feedback control and product inhibition in the activation of factor X by factors IXa and VIII. *Haemostasis*. 1991;21:208-18.
29. Willems GM, Lindhout T, Hermens WT, Hemker HC. Simulation model for thrombin generation in plasma. *Haemostasis*. 1991;21:197-207.
30. Pieters J, Lindhout T, Hemker HC. In situ-generated thrombin is the only enzyme that effectively activates factor VIII and factor V in thromboplastin-activated plasma. *Blood*. 1989;74:1021-4.
31. Nesheim ME, Taswell JB, Mann KG. The contribution of bovine Factor V and Factor Va to the activity of prothrombinase. *J Biol Chem*. 1979;254:10952-62.
32. Langdell RD WR, Brinkhous KM. Effect of hemophillic factor on one stage clotting tests. *J Lab Clin Med*. 1953;41:637.
33. Nye SW GJ, Brinkhous KM. The partial thromboplastin time for the detection of late bleeders. *Am J Med Sci*. 1962;243:279.
34. Quick A GM. Screening for bleeding states- the partial thromboplastin time. *Am J Clin Pathol*. 1963;40:465.
35. Goulian M Bw. The partial thromboplastin time test. Modification of the procedure and a study of the senisivity and optimal conditions. *Am J Clin Pathol*. 1965;44:97.
36. Mant M. J HJ, Pineo GF, Luke KH. Prolonged prothrombin time and partial thomboplastin time in disseminated intravascular coagulation not due to the deficiency of factors V and VIII. *Br J Haematol*. 1973;24:725.
37. Hirsh J. Heparin. *N Engl J Med*. 1991;324:1565-74.
38. Ofosu FA, Sie P, Modi GJ, Fernandez F, Buchanan MR, Blajchman MA, Boneu B, Hirsh J. The inhibition of thrombin-dependent positive-feedback reactions is critical to the expression of the anticoagulant effect of heparin. *Biochem J*. 1987;243:579-88.
39. Hull RD, Raskob GE, Hirsh J, Jay RM, Leclerc JR, Geerts WH, Rosenbloom D, Sackett DL, Anderson C, Harrison L, et al. Continuous intravenous heparin compared with intermittent subcutaneous heparin in the initial treatment of proximal-vein thrombosis. *N Engl J Med*. 1986;315:1109-14.
40. Theroux P, Ouimet H, McCans J, Latour JG, Joly P, Levy G, Pelletier E, Juneau M, Stasiak J, deGuise P, et al. Aspirin, heparin, or both to treat acute unstable angina. *N Engl J Med*. 1988;319:1105-11.
41. Hsia J, Hamilton WP, Kleiman N, Roberts R, Chaitman BR, Ross AM. A comparison between heparin and low-dose aspirin as adjunctive therapy with tissue plasminogen activator for acute myocardial infarction. Heparin-Aspirin Reperfusion Trial (HART) Investigators. *N Engl J Med*. 1990;323:1433-7.
42. Mattox KL, Guinn GA, Rubio PA, Beall AC, Jr. Use of the activated coagulation time in intraoperative heparin reversal for cardiopulmonary operations. *Ann Thorac Surg*. 1975;19:634-8.
43. Bull BS, Huse WM, Brauer FS, Korpman RA. Heparin therapy during extracorporeal circulation. II. The use of a dose-response curve to individualize heparin and protamine dosage. *J Thorac Cardiovasc Surg*. 1975;69:685-9.
44. Young JA, Kisker CT, Doty DB. Adequate anticoagulation during cardiopulmonary bypass determined by activated clotting time and the appearance of fibrin monomer. *Ann Thorac Surg*. 1978;26:231-40.

45. Dougherty KG, Gaos CM, Bush HS, Leachman DR, Ferguson JJ. Activated clotting times and activated partial thromboplastin times in patients undergoing coronary angioplasty who receive bolus doses of heparin. *Cathet Cardiovasc Diagn*. 1992;26:260-3.
46. Ogilby JD, Kopelman HA, Klein LW, Agarwal JB. Adequate heparinization during PTCA: assessment using activated clotting times. *Cathet Cardiovasc Diagn*. 1989;18:206-9.
47. Colman RW, Oxley L, Giannusa P. Statistical comparison of the automated activated partial thromboplastin time and the clotting time in the regulation of heparin therapy. *Am J Clin Pathol*. 1970;53:904-7.
48. Green D, Ts'ao C, Reynolds N, Kahn D, Kohl H, Cohen I. In vitro studies of a new synthetic thrombin inhibitor. *Thromb Res*. 1985;37:145-53.
49. Jakubowski JA, Maraganore JM. Inhibition of coagulation and thrombin-induced platelet activities by a synthetic dodecapeptide modeled on the carboxy-terminus of hirudin. *Blood*. 1990;75:399-406.
50. Jang IK, Gold HK, Ziskind AA, Leinbach RC, Fallon JT, Collen D. Prevention of platelet-rich arterial thrombosis by selective thrombin inhibition. *Circulation*. 1990;81:219-25.
51. Carteaux JP, Gast A, Tschopp TB, Roux S. Activated clotting time as an appropriate test to compare heparin and direct thrombin inhibitors such as hirudin or Ro 46-6240 in experimental arterial thrombosis. *Circulation*. 1995;91:1568-74.
52. Granger CB MD. Bedside anticoagulation testing. In: Topol E, ed. *Acute coronary syndromes*. 2nd ed. New York: Marcel Dekker Inc.; 2001.
53. *Gusto Gazzette*. 1992;July/August:2.
54. Hattersley PG. Activated coagulation time of whole blood. *Jama*. 1966;196:436-40.
55. Colman RW, Bagdasarian A, Talamo RC, Scott CF, Seavey M, Guimaraes JA, Pierce JV, Kaplan AP. Williams trait. Human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman factor-dependent pathways. *J Clin Invest*. 1975;56:1650-62.
56. Schriever HG, Epstein SE, Mintz MD. Statistical correlation and heparin sensitivity of activated partial thromboplastin time, whole blood coagulation time, and an automated coagulation time. *Am J Clin Pathol*. 1973;60:323-9.
57. Avendano A, Ferguson JJ. Comparison of Hemochron and HemoTec activated coagulation time target values during percutaneous transluminal coronary angioplasty. *J Am Coll Cardiol*. 1994;23:907-10.
58. Ferguson J. All ACT's are not the same. *Texas Heart Inst J*. 1992;19.
59. Dietrich W JM. Effect of celite and kaolin on activated clotting time in the presence of aprotinin: Activated clotting time is reduced by binding of aprotinin to kaolin. *J Thorac Cardiovasc Surg*. 1995;109:177.
60. Moliterno DJ, Califf RM, Aguirre FV, Anderson K, Sigmon KN, Weisman HF, Topol EJ. Effect of platelet glycoprotein IIb/IIIa integrin blockade on activated clotting time during percutaneous transluminal coronary angioplasty or directional atherectomy (the EPIC trial). Evaluation of c7E3 Fab in the Prevention of Ischemic Complications trial. *Am J Cardiol*. 1995;75:559-62.

61. Feuillu A, Morel I, Mollard JF. [Evaluation of portable point of care instrument: the i-Stat . Report of 7,000 analyses]. *Ann Biol Clin (Paris)*. 2002;60:153-64.
62. Papadea C, Foster J, Grant S, Ballard SA, Cate JCt, Southgate WM, Purohit DM. Evaluation of the i-STAT Portable Clinical Analyzer for point-of-care blood testing in the intensive care units of a university children's hospital. *Ann Clin Lab Sci*. 2002;32:231-43.
63. Schussler JM, Aguanno JJ, Glover EN, Vish NA, Wissinger LA, Schumacher JR, Wheelan KR. Comparison of the i-STAT handheld activated clotting time with the Hemochron activated clotting time during and after percutaneous coronary intervention. *Am J Cardiol*. 2003;91:464-6.
64. Oberhardt BJ, Dermott SC, Taylor M, Alkadi ZY, Abruzzini AF, Gresalfi NJ. Dry reagent technology for rapid, convenient measurements of blood coagulation and fibrinolysis. *Clin Chem*. 1991;37:520-6.
65. Tsimikas S, Beyer R, Hassankhani A. Relationship between the heparin management test and the HemoTec activated clotting time in patients undergoing percutaneous coronary intervention. *J Thromb Thrombolysis*. 2001;11:217-21.
66. Helft G, Bartolomeo P, Zaman AG, Worthley SG, Chokron S, Le Pailleur C, Beygui F, Le Feuvre C, Metzger JP, Vacheron A, Samama MM. The heparin management test: a new device for monitoring anticoagulation during coronary intervention. *Thromb Res*. 1999;96:481-5.
67. Helft G, Choktron S, Beygui F, Le Feuvre C, Elalamy I, Metzger JP, Vacheron A, Samama MM. Comparison of activated clotting times to heparin management test for adequacy of heparin anticoagulation in percutaneous transluminal coronary angioplasty. *Cathet Cardiovasc Diagn*. 1998;45:329-31.
68. Fitch JC, Geary KL, Mirto GP, Byrne DW, Hines RL. Heparin management test versus activated coagulation time during cardiovascular surgery: correlation with anti-Xa activity. *J Cardiothorac Vasc Anesth*. 1999;13:53-7.
69. Berlex Laboratories W, NJ. Refludan (Lepirudin) package insert. In; 2001.
70. The Medicines Company C, MA. Angimax (bivalirudin) package insert. 2000.
71. Texas Biotechnology Corporation and GlaxoSmithkline H, tx and Philadelphia, PA. Argatroban package insert. 2000.
72. Bates SM WJ. The mechanism of action of thrombin inhibitors. *J Invasive Cardiol*. 2001;12:27F-32.
73. Simko RJ, Tsung FF, Stanek EJ. Activated clotting time versus activated partial thromboplastin time for therapeutic monitoring of heparin. *Ann Pharmacother*. 1995;29:1015-21; quiz 1061.
74. de Denus S, Spinler SA. Clinical monitoring of direct thrombin inhibitors using the ecarin clotting time. *Pharmacotherapy*. 2002;22:433-5.
75. Cannon CP, Maraganore JM, Loscalzo J, McAllister A, Eddings K, George D, Selwyn AP, Adelman B, Fox I, Braunwald E, et al. Anticoagulant effects of hirulog, a novel thrombin inhibitor, in patients with coronary artery disease. *Am J Cardiol*. 1993;71:778-82.
76. Herrman JP, Suryapranata H, den Heijer P, Gabriel L, Kutryk MJ, Serruys PW. Argatroban During Percutaneous Transluminal Coronary Angioplasty: Results of a Dose-Verification Study. *J Thromb Thrombolysis*. 1996;3:367-375.

77. Moser M, Ruef J, Peter K, Kohler B, Gulba DC, Paterna N, Nordt T, Kubler W, Bode C. Ecarin clotting time but not aPTT correlates with PEG-hirudin plasma activity. *J Thromb Thrombolysis*. 2001;12:165-9.
78. Nowak G BE. Quantitative determination of hirudin in blood and body fluids. *Semin Thromb Hemost*. 1996;22:197-202.
79. Potzsch B, Hund S, Madlener K, Unkrig C, Muller-Berghaus G. Monitoring of recombinant hirudin: assessment of a plasma-based ecarin clotting time assay. *Thromb Res*. 1997;86:373-83.
80. Potzsch B, Madlener K, Seelig C, Riess CF, Greinacher A, Muller-Berghaus G. Monitoring of r-hirudin anticoagulation during cardiopulmonary bypass--assessment of the whole blood ecarin clotting time. *Thromb Haemost*. 1997;77:920-5.
81. Diagnostic. C. TAS ECT test card package insert. In: Raleigh N, ed.; 2000.
82. Cho L, Kottke-Marchant K, Lincoff AM, Roffi M, Reginelli JP, Kaldus T, Moliterno DJ. Correlation of Point-of-Care ecarin clotting time versus activated clotting time with bivalirudin concentrations. *Am J Cardiol*. 2003;91:1110-3.
83. Nowak G. Clinical monitoring of hirudin and direct thrombin inhibitors. *Semin Thromb Hemost*. 2001;27:537-41.
84. Hartert H. Blutgerinnungsstudien mit der Thrombelatographie, einem neuen Untersuchungsverfahren. *Klin Wochenscgr*. 1948;26:577-583.
85. Mallett SV, Cox DJ. Thrombelastography. *Br J Anaesth*. 1992;69:307-13.
86. Wenker O, Wojciechowski Z, Sheinbaum R, Zisman E. Thrombelastography. *The Internet Journal of Anesthesiology*. 2000;1.
87. Pivalizza EG. Monitoring of hirudin therapy with the Thrombelastograph. *J Clin Anesth*. 2002;14:456-8.
88. Srinivasa V, Gilbertson LI, Bhavani-Shankar K. Thromboelastography: where is it and where is it heading? *Int Anesthesiol Clin*. 2001;39:35-49.
89. Tuman KJ, Spiess BD, McCarthy RJ, Ivankovich AD. Comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. *Anesth Analg*. 1989;69:69-75.
90. Kang YG, Martin DJ, Marquez J, Lewis JH, Bontempo FA, Shaw BW, Jr., Starzl TE, Winter PM. Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. *Anesth Analg*. 1985;64:888-96.
91. Shore-Lesserson L, Manspeizer HE, DePerio M, Francis S, Vela-Cantos F, Ergin MA. Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. *Anesth Analg*. 1999;88:312-9.
92. Spiess BD, Tuman KJ, McCarthy RJ, DeLaria GA, Schillo R, Ivankovich AD. Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. *J Clin Monit*. 1987;3:25-30.
93. Gorton H LG. Thromboelastography and low molecular weight therapy in pregnancy. *Anesthesiology*. 1999;90.
94. Steer PL. Anaesthetic management of a parturient with thrombocytopenia using thrombelastography and sonoclot analysis. *Can J Anaesth*. 1993;40:84-5.

95. Steer PL FB, Blumenthal LA. Abruptio placentae and disseminated intravascular coagulation, use of thromboelastography and sonoclot analysis. *Int J Obstet Anesth*. 1994;3:229-233.
96. Shenaq SA SA. *Viscoelastic measurment of clot formation: The Sonoclot.* Philadelphia: WB Saunders Company; 1988.
97. Hett DA, Walker D, Pilkington SN, Smith DC. Sonoclot analysis. *Br J Anaesth*. 1995;75:771-6.
98. Sonoclot coagulation & platelet function analyzer: An overview (Product monograph). In: *Sienco, Inc., CO,USA*.
99. Furuhashi M, Ura N, Hasegawa K, Yoshida H, Tsuchihashi K, Miura T, Shimamoto K. Sonoclot coagulation analysis: new bedside monitoring for determination of the appropriate heparin dose during haemodialysis. *Nephrol Dial Transplant*. 2002;17:1457-62.
100. Francis JL, Francis DA, Gunathilagan GJ. Assessment of hypercoagulability in patients with cancer using the Sonoclot Analyzer and thromboelastography. *Thromb Res*. 1994;74:335-46.
101. Pharmanetics Inc. M, NC, USA. ENOX test card Package insert. 2002.
102. Mize P CG, Oliver J, Stallings P, Leumas J, Mahan D, DeAnglis A. Development of the Rapidpoint™ Coag Enoxaparin Test Card System to Monitor Lovenox® (enoxaparin sodium) in PCI patients. *Blood*. 2001;98:185a.
103. Mize P CG, Oliver J, Stallings P, Leumas J, Mahan D, DeAnglis A. Rapidpoint™ Coag Enoxaparin Test: Clinical results for a POC Method for Monitoring Lovenox® (enoxaparin sodium) in Patients Undergoing PCI. *Blood*. 2001;98:193a.
104. Kereiakes DJ, Grines C, Fry E, Esente P, Hoppensteadt D, Midei M, Barr L, Matthai W, Todd M, Broderick T, Rubinstein R, Fareed J, Santoian E, Neiderman A, Brodie B, Zidar J, Ferguson JJ, Cohen M. Enoxaparin and abciximab adjunctive pharmacotherapy during percutaneous coronary intervention. *J Invasive Cardiol*. 2001;13:272-8.
105. Young JJ, Kereiakes DJ, Grines CL. Low-molecular-weight heparin therapy in percutaneous coronary intervention: the NICE 1 and NICE 4 trials. National Investigators Collaborating on Enoxaparin Investigators. *J Invasive Cardiol*. 2000;12 Suppl E:E14-8;discussion E25-8.
106. Collet JP, Montalescot G, Lison L, Choussat R, Ankri A, Drobinski G, Sotirov I, Thomas D. Percutaneous coronary intervention after subcutaneous enoxaparin pretreatment in patients with unstable angina pectoris. *Circulation*. 2001;103:658-63.
107. Braunwald E AE, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith EE 3rd, Steward DE, Theroux P, Gibbons RJ, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Hiratzka LF, Jacobs AK, Smith SC Jr. ACC/AHA guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction--2002: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *J Am Coll Cardiol*. 2002;40:1366-74.

108. Buchanan MR, Brister SJ. Individual variation in the effects of ASA on platelet function: implications for the use of ASA clinically. *Can J Cardiol*. 1995;11:221-7.
109. Helgason CM BK, Hoff JA, Winkler SR, Mangat A, Tortorice KL, Brace LD. Development of aspirin resistance in persons with previous ischemic stroke. *Stroke*. 1994;25:2331-2336.
110. Thompson CM, Steinhubl SR. Monitoring of platelet function in the setting of glycoprotein IIb/IIIa inhibitor therapy. *J Interv Cardiol*. 2002;15:61-70.
111. Coller BS, Lang D, Scudder LE. Rapid and simple platelet function assay to assess glycoprotein IIb/IIIa receptor blockade. *Circulation*. 1997;95:860-7.
112. Smith JW, Steinhubl SR, Lincoff AM, Coleman JC, Lee TT, Hillman RS, Coller BS. Rapid platelet-function assay: an automated and quantitative cartridge-based method. *Circulation*. 1999;99:620-5.
113. Coller BS, Folts JD, Smith SR, Scudder LE, Jordan R. Abolition of in vivo platelet thrombus formation in primates with monoclonal antibodies to the platelet GPIIb/IIIa receptor. Correlation with bleeding time, platelet aggregation, and blockade of GPIIb/IIIa receptors. *Circulation*. 1989;80:1766-74.
114. Tcheng JE, Ellis SG, George BS, Kereiakes DJ, Kleiman NS, Talley JD, Wang AL, Weisman HF, Califf RM, Topol EJ. Pharmacodynamics of chimeric glycoprotein IIb/IIIa integrin antiplatelet antibody Fab 7E3 in high-risk coronary angioplasty. *Circulation*. 1994;90:1757-64.
115. Casterella P KD, Steinhubl SR et al. coronary intervention. Use of the rapid platelet function analyzer (RPFA) to evaluate platelet function in patients receiving incremental bolus dosing of abciximab during percutaneous. *J Am Coll Cardiol*. 1999;33:39A.
116. Steinhubl SR, Kottke-Marchant K, Moliterno DJ, Rosenthal ML, Godfrey NK, Coller BS, Topol EJ, Lincoff AM. Attainment and maintenance of platelet inhibition through standard dosing of abciximab in diabetic and nondiabetic patients undergoing percutaneous coronary intervention. *Circulation*. 1999;100:1977-82.
117. Steinhubl SR, Talley JD, Braden GA, Tcheng JE, Casterella PJ, Moliterno DJ, Navetta FI, Berger PB, Popma JJ, Dangas G, Gallo R, Sane DC, Saucedo JF, Jia G, Lincoff AM, Theroux P, Holmes DR, Teirstein PS, Kereiakes DJ. Point-of-care measured platelet inhibition correlates with a reduced risk of an adverse cardiac event after percutaneous coronary intervention: results of the GOLD (AU-Assessing Ultegra) multicenter study. *Circulation*. 2001;103:2572-8.
118. Mammen EF, Comp PC, Gosselin R, Greenberg C, Hoots WK, Kessler CM, Larkin EC, Liles D, Nugent DJ. PFA-100 system: a new method for assessment of platelet dysfunction. *Semin Thromb Hemost*. 1998;24:195-202.
119. Nicholson NS, Panzer-Knodle SG, Haas NF, Taite BB, Szalony JA, Page JD, Feigen LP, Lansky DM, Salyers AK. Assessment of platelet function assays. *Am Heart J*. 1998;135:S170-8.
120. Fressinaud E VA, Truchaud F, Martin I, Boyer-Neumann C, Trossaert M, Meyer D. Screening for von Willebrand disease with a new analyzer using high shear stress: a study of 60 cases. *Blood*. 1998;91:1325-1331.

121. Macik BG. Designing a point-of-care program for coagulation testing. *Arch Pathol Lab Med*. 1995;119:929-38.
122. Colman RW CA, George JN, Hirsh J, Marder VJ. Overview of hemostasis. In: Colman RW HJ, Marder VJ, Clowes AW, George JN, ed. *Hemostasis and thrombosis*. Fourth ed: Lippincott Williams & Wilkins; 2001.
123. Ansell J, Tiarks C, Hirsh J, McGehee W, Adler D, Weibert R. Measurement of the activated partial thromboplastin time from a capillary (fingerstick) sample of whole blood. A new method for monitoring heparin therapy. *Am J Clin Pathol*. 1991;95:222-7.
124. Becker RC, Ball SP, Eisenberg P, Borzak S, Held AC, Spencer F, Voyce SJ, Jesse R, Hendel R, Ma Y, Hurley T, Hebert J. A randomized, multicenter trial of weight-adjusted intravenous heparin dose titration and point-of-care coagulation monitoring in hospitalized patients with active thromboembolic disease. Antithrombotic Therapy Consortium Investigators. *Am Heart J*. 1999;137:59-71.
125. Ruzicka K, Kapiotis S, Quehenberger P, Handler S, Hornykewycz S, Michitsch A, Huber K, Clemens D, Susan M, Pabinger I, Eichinger S, Jilma B, Speiser W. Evaluation of bedside prothrombin time and activated partial thromboplastin time measurement by coagulation analyzer CoaguCheck Plus in various clinical settings. *Thromb Res*. 1997;87:431-40.
126. Brill-Edwards P GJ, Johnston M, Hirsh J. Establishing a therapeutic range for heparin therapy. *Ann Intern Med*. 1993;119:104-109.

Figure Legends:

1. **The Coagulation cascade.** The central precipitating event is considered to involve tissue factor (TF), which under physiological conditions is not exposed to blood. With vascular or endothelial cell injury, TF acts in concert with activated factor VIIa and phospholipids (PL) to convert factor IX to IXa and X to Xa. The intrinsic pathway includes 'contact' activation of factor XI by factor XIIa/ activated high-molecular weight-kininogen (HKa) complex. It should be noted that the contact system contributes to fibrinolysis and bradykinin formation in vivo. Factore XIa also converts factor IX to IXa and factor IXa in turn converts factor X to Xa, in concert with factor VIII a and PL (the tenase complex). Fqactor Xa is the catalytic ingredient of the prothrombinase complex and converts prothrombin to thrombin. Thrombin cleaves the fibrinopeptides (FPA, FPB) from fibrinogen, allowing the resultant fibrin monomers to polymerize, and converts factor XIII to XIIIa, which crosslinks (XL) the fibrin clot. Thrombin accelerates the process (interrupted lines) by its potential to activate factors V and VIII, but continued proteolytic process also dampens the process by activating protein C, which degrades factors Va and VIIIa. Natural plasma inhibitors retard clotting: C1-inhibitor (C1 INH) neutralizes factor XIIa, tissue factor pathway inhibitor (TFPI) blocks factor Viia/TF, and antithrombin (ATIII) blocks factors IXa and Xa and thrombin. (Arrows, active enzymes; filled rectangles, sites of inhibitor action; dashed lines., feedback reactions.) From reference[122]

2. **Fibrinopolymer detection methods**. A: Light transmission of a plasma sample increases as fibrinogen leaves the solution in fibrin polymers. A threshold value or percentage of light transmission may be set to stop the timer. B: As fibrin polymers form, specimen viscosity rises. When a plunger is lifted and then allowed to fall, the rate of fall is a measure of specimen viscosity. C: Two electrodes may be placed in a specimen and a current established. One of the electrodes may be withdrawn from the specimen at preset

intervals. When viscosity rises, contact between the moving electrode and the specimen will not be broken and the timer will stop. D: If a ferromagnetic specimen is placed in a specimen, motion may be established by an oscillating magnetic field. When motion is restricted to a preset value, timing will stop. E: A probe may be placed in the specimen and vibrated at a preset frequency. A sensor positioned outside the specimen, regulates the output of the probe to maintain a constant value. Fibrin polymerization and increased viscosity decrease sound transmission, increasing the output required of the probe.

3. **The internal mechanism of the Rapidpoint Coag® heparin management test (dry reagent technology)**. Above the platform for the test card is a photo-detector and a photo-diode. Below the platform are the bias and electro magnets which, during the test procedure, influence the PIOP to move. As a clot begins to form, fibrin strands attach themselves to the particles, impeding their movement. Movement slows until the particles are entrapped in the clot and stop moving. The particle movement is monitored optically as changes in reflected light from the surface of the test card. When the movement stops no light changes are seen and the test is complete. For fibrinolysis tests the process is reversed. When a sample is added to the test card a clot is formed immediately and the particles are trapped. The analyzer monitors time for the clot to dissolve and free the particles to begin moving. (From Cardiovascular Diagnostics, INC., Raleigh, N.C.)

4. **Correlation between ECT, ACT and bivalirudin levels**. Considering samples from all time points, the correlations between clotting time and bivalirudin concentration were r=0.90 for ECT and r=0.71 for Hemochron ACT. The ellipse shown on each graph represents the 90% density contour (confidence curve for the bivariate distribution). The ellipse becomes more circular in shape as the correlation decreases. From reference[82].

5. **The Thromboelastograph**® (Hemoscope, Morton Grove, Ill). See text for details.

6. **Thromboelastography tracing parameters**. See text for details. (From Hemoscope, Skokie, IL)

7. **Characteristic thromboelastograph tracings.** Modified from reference [88]

8**. Representative description of the the Sonoclot Signature and variables**. From reference[99]. See text for details.

Table 1. Point of care devices for determination of aPTT

	Instrument		
	Coagucheck Plus[123,124]08	Hemochron Jr.	Thrombolytic Assesment system (TAS)[64]
Manufacturer	Roche & Boehringer-Mannheim Diagnostics	International Technidyne Corp.	Cardiovascular Diagnostics Inc.
Blood sample	Fresh whole blood	Whole blood or citrated plasma	Citrated whole blood or plasma
Sample volume	1 drop	1 drop	1 drop
Time to result	Minutes	Minutes	Minutes
Setting of clot formation	Capillary flow of unclotted blood	Forced movement of unclotted blood through a narrow channel	Chamber with magnetic particles with oscillating electric field
Clot detection method	Laser photometry	Optical light transmission	Optical light transmission
Correlation with standard laboratory	r =0.78-0.89[125,126]10	R = 0.87	R = 0.84[121]11
Therapeutic aPTT[a]	60-85 sec (GUSTO III)	50-70 sec (PARAGON)	Customized to each hospitals standard aPTT range

Modified from reference 52

[a] Corresponding to heparin level of 0.2-0.4U/ml

Table 2. Salient features of point-of-care whole blood coagulation analyzers

	Hemochron	***HemoTec***	***TAS/HMT***
Manufacturer	International Technidyne Corp Edison, NJ	Medtronic, Inc, Englewood, CO	Cardiovascular Diagnostics, Inc, Raleigh, NC
Amount of blood	2ml	0.2-0.4ml	1 drop (35µl)
Contact activator	Celite	Kaolin	celite
Testing vehicle	Test tube	Test cartridge	Test card
Sensor method	Electro-mechanical	Electro-mechanical	Electro-optical
Detection system	Magnet in test tube	Plastic flag	Iron oxide particles on test card
Data management/storage	No	No	Yes

Modified from reference [65]

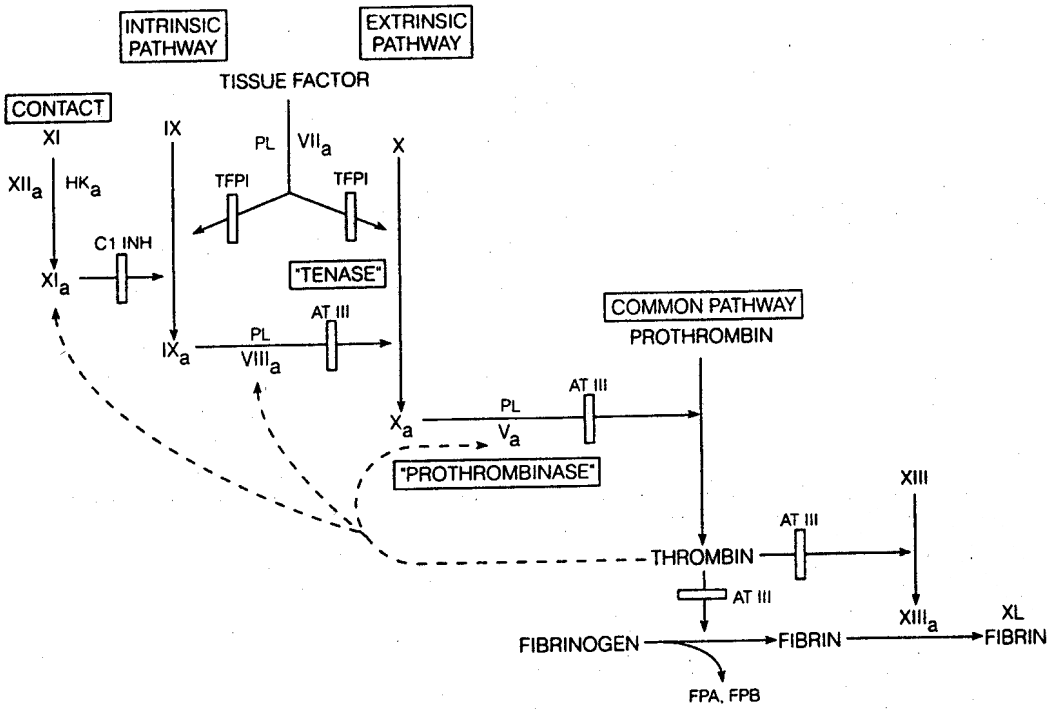

Figure 1.

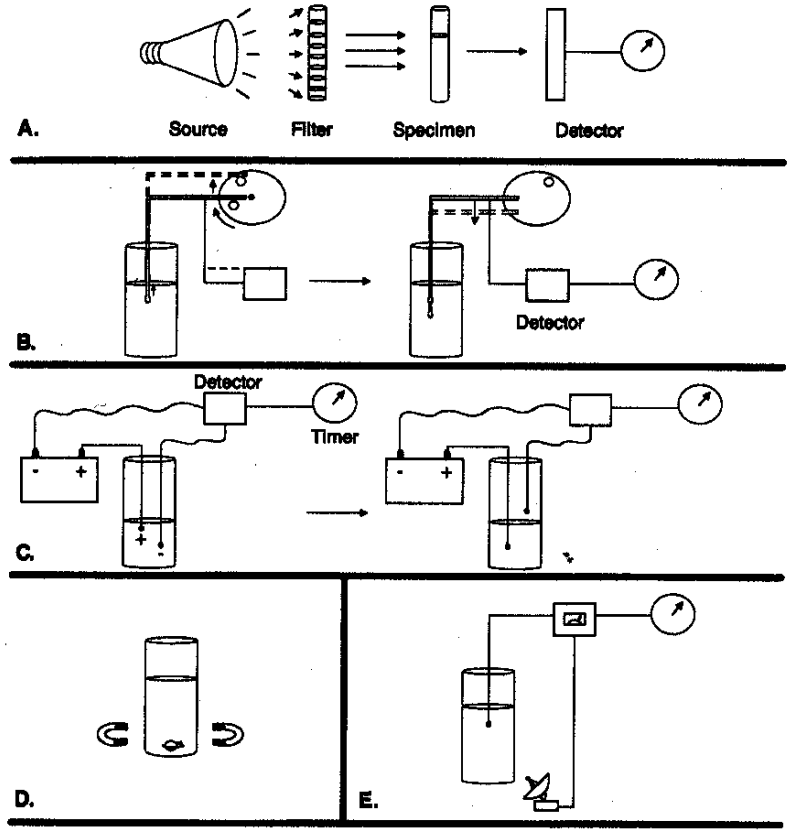

Figure2

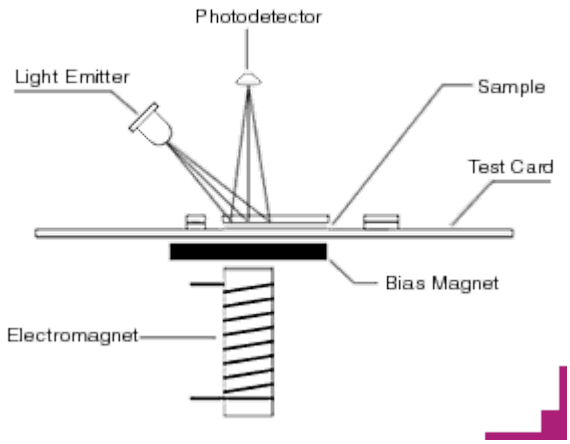

Figure 3

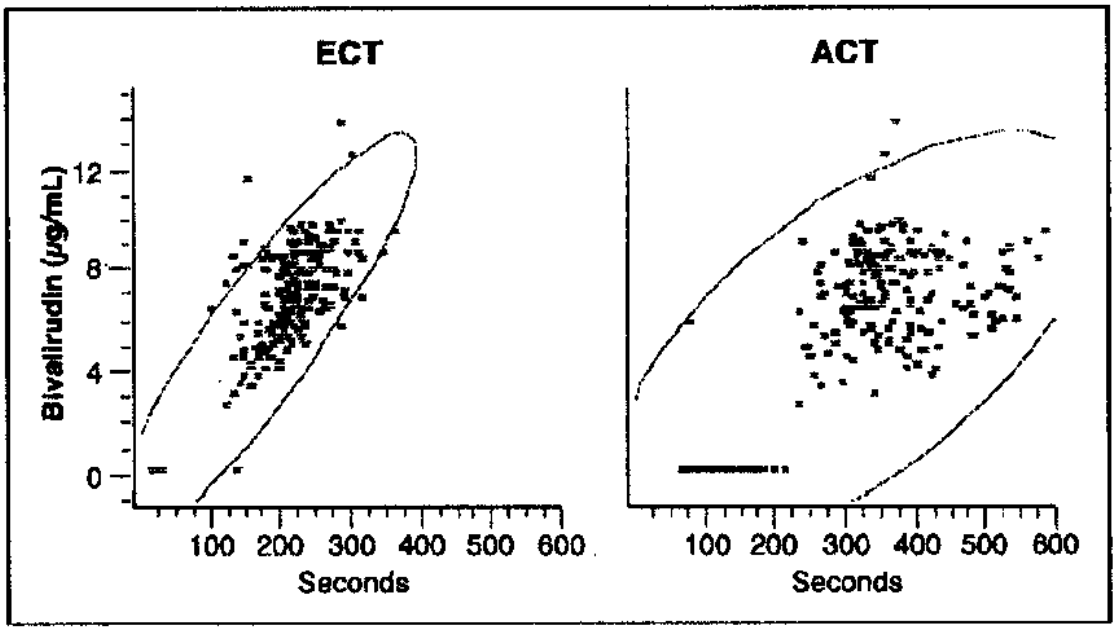

Figure 4

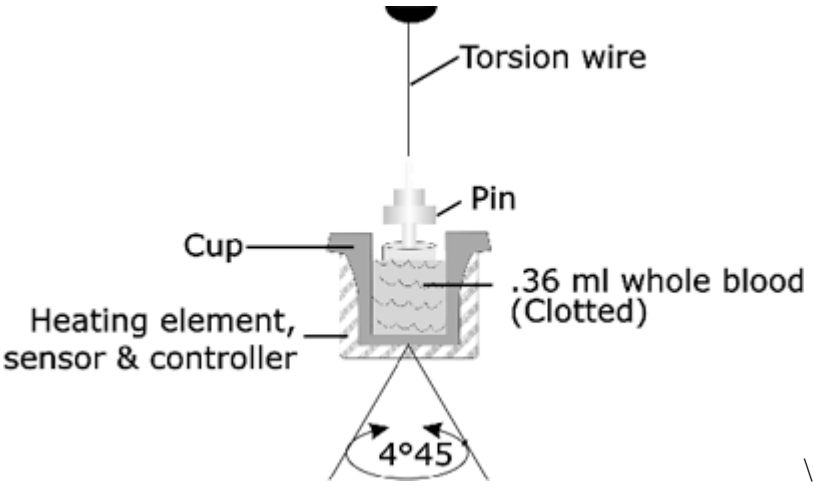

FIGURE 5

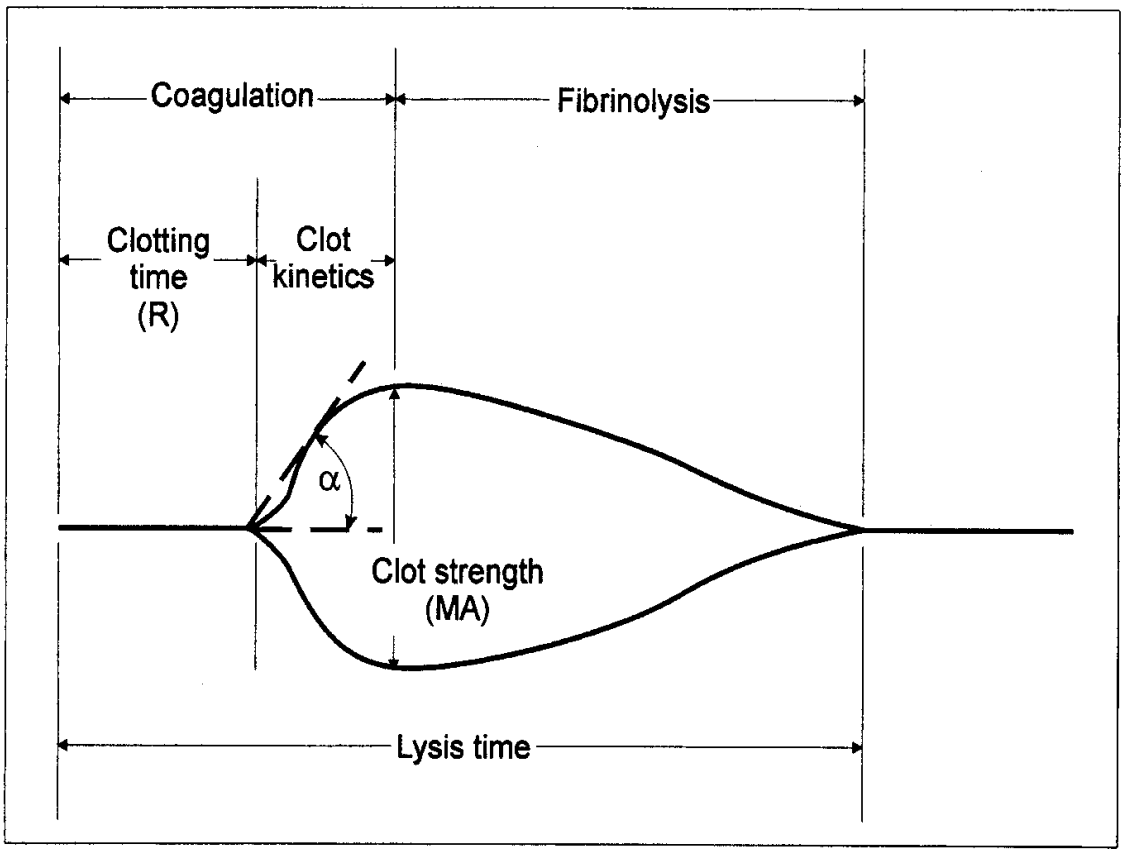

Figure 6

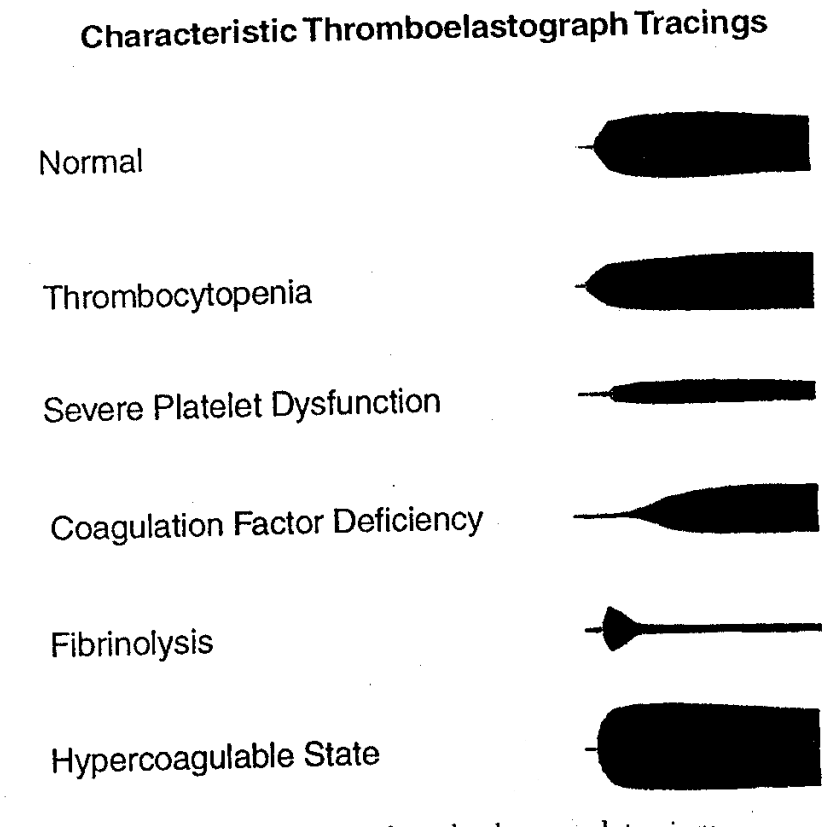

Figure 7

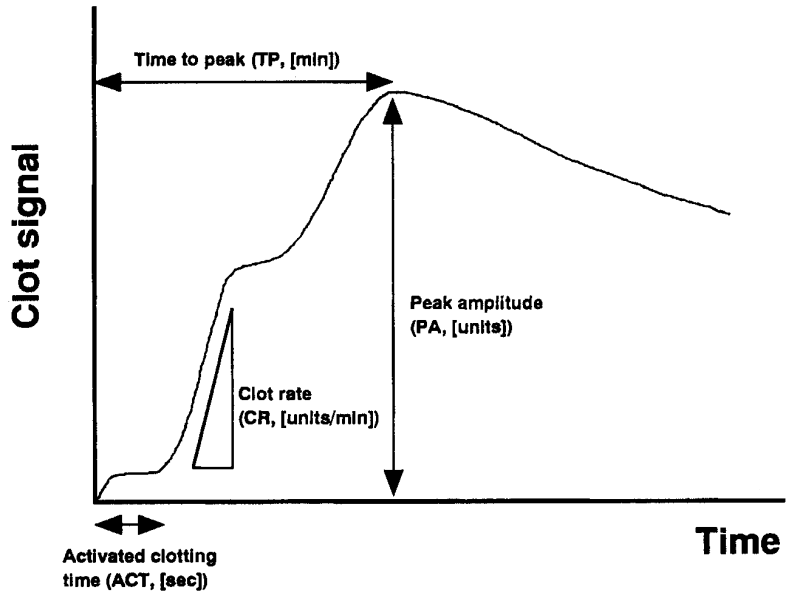

Figure 8