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Thromboelastography for Evaluation of Coagulation Status: A Description of the Method and Discussion of Case Examples

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Thromboelastography (TEG) is a diagnostic method for evaluation of coagulation status that provides a dynamic view of clot development and fibrinolysis in a sample of whole blood. Viscoelastic changes in the blood are detected as a clot develops and a continuous tracing is generated that provides information regarding the time to initial fibrin formation, the kinetics of clot development, maximum clot strength and rate of fibrinolysis. By comparison, standard coagulation tests such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT) are performed on platelet-poor plasma and report an endpoint which is the time to initial formation of cross-linked fibrin. Because TEG provides a continuous assessment of coagulation that includes contributions both from cells and plasma proteins, this method may provide a superior model for in vivo hemostasis.

Thrombelastography was first described by Hartert in the late 1940s. In recent years TEG has become a more accessible diagnostic tool as reagents and instruments have been developed that provide standardized analysis with computer-generated recording and evaluation of results. Two instruments are currently available that employ this methodology. This report will focus on the Thrombelastograph (TEG; Haemoscope Corporation) which is used most commonly in this country. A second analyzer (ROTEM, rotational thromboelastometry; Pentapharm GmbH) differs in the strategy that is used to detect clot formation but gives similar information, and is currently used primarily in Europe. A detailed comparison of these two instruments is provided in a recent review.

TEG can be performed as a patient-side test using fresh whole blood, but in veterinary medicine this test typically uses blood anticoagulated with sodium citrate. Clot formation is initiated by recalcification of the sample at a defined time after blood collection, and standardization of the time period between sample collection and performance of the assay is critical for the interpretation of results. Activators of coagulation such as kaolin or tissue factor (TF) can be employed to decrease the time required to generate a tracing and may decrease analytical variation. As for other coagulation tests, blood should be collected atraumatically and transported to the diagnostic laboratory without delay.

Citrated whole blood is held at room temperature for a defined period of time that is chosen by the individual laboratory. To perform the assay, 340 uL blood is placed in a cup that is pre-warmed to 37°C and contains 20 uL of 0.2 M calcium chloride. The cup is raised so that a pin connected to a torsion wire sits within the blood. The cup oscillates slowly back and forth, and initially the blood moves freely around the pin. As the blood sample clots, fibrin strands form between the pin and the wall of the cup. This causes movement of the pin with the cup, which is detected by the torsion wire and
transmitted to the computer where it is converted into a tracing that gives an indication of clot strength throughout the coagulation process. (Figure 1)

**Figure 1.** The TEG analyzer can be used to analyze two samples simultaneously (analysis of a given sample is usually performed in duplicate). For each, a disposable cup is placed within a heating element so that the reaction proceeds at a controlled temperature. The sample is added to the cup, a disposable pin is located within the sample and recording begins. Movement of the pin is detected by the torsion wire and is used to generate a tracing that indicates the extent of clot formation.

Information derived from the TEG tracing includes the time to initial fibrin formation (reaction time; R), the time needed to reach a predetermined clot strength (clotting time; K), the rate of clot formation (angle; $\alpha$), and maximum clot strength (MA). MA is the maximum amplitude of the tracing measured in millimeters, and can be converted to G, which expresses clot strength in units of force (dynes/cm²). (Figure 2) Prolonged data collection provides information regarding fibrinolysis.

**Figure 2.** Elapsed time in minutes is indicated on the x-axis. The y-axis shows the amplitude of pin movement in millimeters. R (minutes) is the time from the start of recording until the tracing reaches an amplitude of 2 mM. K (minutes) is the time for the tracing amplitude to increase from 2 to 20 mM. $\alpha$ - angle (degrees) is based on a line drawn tangential to the tracing between R and K. MA (millimeters) is the maximum amplitude of the tracing. G (dynes/cm²) is derived from MA using the formula: $G = \frac{(5000 \times MA)}{(100 - MA)}$. 

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Many studies have examined the association between standard coagulation assay findings and TEG results, and a complete review of that work is beyond the scope of this paper. R appears to be affected primarily by plasma coagulation factor activity, and has shown a direct correlation with the PT and/or the aPTT in some, but not all studies. K and the α-angle are complex values that are dependent on coagulation factor activity, fibrinogen concentration and platelets. MA is determined primarily by platelet number and function together with fibrinogen concentration, and there is generally a strong correlation between MA and those factors. Additional contributions to clot strength come from other factors related to fibrin formation.

TEG has been validated for use as a diagnostic test in dogs, cats and horses. A major point of difference between reports is the decision of whether or not to use a coagulation activator, and what type of activator is employed. Native TEG is performed when the blood is recalcified without addition of a coagulation activator. Activators that may be used include kaolin (Haemoscope) or human recombinant TF (Innovin, Dade Behring). Kaolin is a commercially available reagent that is provided in pre-measured amounts, while the TF reagent must be diluted prior to each use. The advantage of native TEG is that it is slightly less expensive and requires less manipulation of the sample and reagents. Activators have the potential to decrease the lag time before initial fibrin formation and may also decrease analytical variation, although the magnitude of this effect varies in different reports. In our laboratory we compared the results of native TEG and TEG activated with kaolin in healthy dogs and did not find any significant differences, but we have not made a similar comparison of the results found in sick dogs. TEG does not require any additional sample preparation and is fairly simple to initiate. Once TEG is started, most results are available within 20-30 minutes. Because a standardized protocol for TEG has not been accepted, it is critical for each laboratory to establish independent reference intervals.

TEG provides a global view of coagulation status and may be more clinically relevant than the results of individual tests that provide an isolated evaluation of specific elements of blood coagulation. In human medicine, TEG is often used to guide the administration of blood products and to predict the risk of hemorrhage associated with surgery. Similar applications may be useful in veterinary patients, and the finding of a hypocoagulable TEG tracing (based on G) in dogs was shown to correlate with clinical signs of hemorrhage in one study. A unique feature of TEG is the ability to detect hypercoagulable states, and the clinical importance of this capability continues to receive a great deal of attention. TEG findings that are indicative of hypercoagulability most often include an increase in the rate of clot accumulation (decreased K, increased angle) and an increase in maximum clot strength (increased MA). Transient hypercoagulable states are often associated with major surgery in people, and TEG may help to predict the likelihood of postoperative thrombosis.

When a group of dogs admitted to an intensive care unit with a wide variety of underlying problems was evaluated, many (52%) had an abnormal TEG tracing and the majority of those were hypercoagulable. Conventional coagulation tests have shown that abnormalities of hemostasis are often present in dogs with cancer, and when TEG was used to evaluate hemostasis in a population of dogs with malignant neoplasia, the
The most common abnormality was hypercoagulability which was found in 50% of the dogs. Dogs with neoplasia have many potential risk factors for thromboembolism, and neoplasia is one of the most common disease processes found in dogs with pulmonary thromboembolism. Thromboembolism is also a frequent complication in dogs with immune-mediated hemolytic anemia, and studies of TEG in dogs with IMHA show that most are hypercoagulable. Prospective studies are needed to determine if TEG findings can be used to predict the risk of thromboembolism in veterinary patients, and if TEG is a useful guide for anticoagulant therapy.

The effect of heparin on TEG can be evaluated by comparing the results of standard TEG to those obtained using a cup that contains the enzyme heparinase (Haemoscope). TEG is quite sensitive to the effects of heparin and it has been shown that administration of the accepted target dose, based on prolongation of the aPTT or anti-Xa activity, results in marked prolongation of R. However TEG can be used to assess efficacy of a given heparin dose in a particular patient, and may help to guide changes in the dosing regimen. Further studies are needed to assess whether lower doses of heparin, based on changes to the TEG tracing, have a positive effect on patient outcome. If this is found to be true, then normalization of the TEG tracing may be a useful therapeutic target.

Standard TEG is not useful for evaluation of the response to platelet function inhibitors that target specific pathways of platelet activation, such as clopidogrel (Plavix), because thrombin produced during coagulation results in strong platelet activation and overrides the effects of these drugs. A modified version of TEG (Platelet mapping; Haemoscope) has been developed that uses blood collected with heparin to eliminate thrombin activity. Reptilase and Factor XIII are used to generate a cross-linked fibrin clot, and platelet activation is initiated by the addition of agonists such as ADP or arachidonic acid. Maximum hemostatic activity of the blood sample is measured in citrated blood activated with kaolin, and the combined results of these tests are used to calculate platelet function. This approach has been used in dogs to assess the effects of clopidogrel, but clinical application of this method in veterinary patients will likely be limited by cost.

Platelets make a substantial contribution to clot strength measured by TEG, but the sensitivity of TEG to specific platelet function defects is variable. Because TEG is performed under low shear conditions, it is not sensitive to defects in platelet adhesion or von Willebrand factor deficiency. Maximum clot strength is substantially decreased in animals with Glanzmann thrombasthenia, in which a decrease in the platelet glycoprotein IIb/IIIa complex impairs platelet aggregation and clot retraction.

Work in our laboratory has included efforts to obtain more basic information regarding this assay method, including further assessment of the impact of time after blood collection, and stability of results when TEG is repeated in individual dogs over several days. We have confirmed the finding of hypercoagulability in dogs with IMHA, and in future studies we hope to characterize the mechanism for that abnormality and assess the clinical impact of treatment given to address that problem. TEG has also been
employed in a research study designed to assess the impact of various formulations of
hetastarch on coagulation status in dogs (Lenore Bacek, personal communication).

TEG does not replace conventional tests of coagulation, such as the PT, aPTT, AT, D-
dimer and fibrinogen concentration, but can augment the information obtained from
those traditional assays and strengthen our ability to assess overall coagulation status.
TEG can help to increase our awareness of the risk of either hemorrhage or
thromboembolism in patients and can be used to monitor the effects of treatment on
hemostasis. In the future, TEG may help to guide both the use of blood products and
the administration of anticoagulant medications in veterinary patients. Normalization of
the TEG tracing may be a sensitive goal that can be used to optimize individual patient
therapy.

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