Thromboelastography in Veterinary Medicine

Bo Wiinberg, D.V.M., Ph.D.,1 and Annemarie T. Kristensen, D.V.M., Ph.D.1

ABSTRACT

Thromboelastography (TEG) has been used in experimental animal studies since the early 1960s and in a routine clinical setting for the past decade. From the data currently available, it is clear that both the scope and limitations of TEG in animals resemble those observed in humans. TEG has been used to diagnose hypercoagulability in animals with disseminated intravascular coagulation, various types of cancer, and critical illness. Its ability to detect and monitor animals with various types of coagulopathies has been well established, both clinically and in experimental studies. TEG is often used in animals to monitor the effect of different pro- and anticoagulant drugs and often performs better at this task than conventional coagulation assays. TEG is already well established in veterinary medicine, and with the rapid dissemination of the technique currently taking place, we can expect to see a wide variety of interesting animal data published in the near future.

KEYWORDS: Hemostasis, TEG, ROTEM, canine, feline, mouse, equine, rabbit, pig

The introduction of the cell-based model of hemostasis has made it evident that hemostasis is influenced by numerous pro- and anticoagulant components of the blood other than those present in the plasma fraction alone. In particular, tissue factor (TF) expression in certain types of tissue and cellular components of the blood such as the activated platelets and leukocytes supply a surface for initiation, amplification, and propagation of clot formation and thus play a key role in hemostasis.1–5 These cellular and tissue components are themselves influenced by altered systemic inflammatory and immune responses during disease.6–9 Because whole blood contains all the intravascular factors and cells participating in physiological and pathological hemostasis, incorporating TF and phospholipid-bearing cells, whole blood–based assays such as thromboelastography (TEG) may provide a more truthful reflection of in vivo hemostasis than the traditionally used plasma-based hemostasis assays in the diagnosis and monitoring of animals with abnormal hemostasis.

TEG has been used in veterinary medicine since the early 1960s when numerous publications reporting its use, especially from Eastern Europe, the Soviet Republic, and Germany, became available. The total number of publications on the use of TEG in animals is surprisingly large and includes >500 publications over the past 50 years. This review focuses primarily on the recent use of TEG in animals, and selected references are used to exemplify clinical and experimental research.

Both the commercial TEG systems on the market, the ROTEM (Rotation Thromboelastometer; Pentapharm GmbH, Munich, Germany) and the TEG (Thromboelastograph; Haemoscope Corp., Niles, IL), have been used in animals. Both machines function on the same principle, namely the gradual binding of a pin to the sides of a cup during the clot formation. The main

1The Small Animal Hospital, Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark.

Address for correspondence and reprint requests: Bo Wiinberg, D.V.M., Ph.D., Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, 16 Dyrlægevej, DK-1870 Frederiksberg C., Denmark (e-mail: bwi@life.ku.dk).
difference between them is that in the TEG, it is the cup that oscillates, and in the ROTEM, it is the pin; therefore, unless otherwise stated, the term thromboelastography is used interchangeably throughout this review.

In veterinary medicine, TEG analyses are almost always performed on citrated whole blood samples, but plasma samples can be assayed as well. Three types of standard TEG assays have been evaluated in veterinary medicine: native (no activator), human recombinant tissue factor activated (INNOVIN; Siemens Healthcare Diagnostics, Deerfield, IL), and kaolin activated (Kaolin; Haemonetics Corp., Braintree, MA). The assay most often used in animals is the native assay, no doubt because it is the simplest and most inexpensive way to perform a TEG analysis, and it offers the opportunity to evaluate the blood clotting process without the interference of any form of exogenous substance. The obvious disadvantage of the native assay is that there is a prolonged lag time from initiation of the assay until first clot formation, which varies considerably among species. Furthermore there are indications of a possible issue of increased analytical variation compared with activated assays, although this has yet to be confirmed in general.

To reduce lag time, increase sensitivity, and minimize analytical variation, activators have been applied in veterinary medicine. Kaolin is a commercially available ready-to-use reagent that is used to reflect the ability of blood clotting initiated via the contact (“intrinsic”) coagulation cascade. TF, in contrast, reflects the ability of blood clotting initiated via the TF (“extrinsic”) coagulation cascade. The appealing aspect of TF is that it potentially reflects the physiological initiation of coagulation as it is perceived to take place in the cell-based model of hemostasis. Unfortunately the TF assay is not commercially available and therefore somewhat more laborious to perform because the reagent has to be prediluted. Regardless of which activator and reagent is used, it is well established that the reference ranges are not interchangeable at the present time, and therefore each laboratory has to establish its own reference range individually.

Established Reference Ranges and Analytical Variation

Although TEG analyses have been performed in a wide range of animal species, only a limited number of studies in animals have aimed at establishing normal values for TEG parameters in healthy individuals (Table 1). This is rarely a problem in experimental studies, where the use of controls groups is encouraged. The challenge arises in the clinical use of TEG in veterinary medicine, and consequently at the present time, each laboratory must establish its own reference ranges and clinically relevant cut-off values.

Normal values in dogs have been established for native TEG, kaolin-activated TEG, and diluted recombinant human TF (rhTF)-activated TEG. The results clearly show that the reference ranges are different and thus not interchangeable. In horses both native and rhTF-activated assays have been validated. One study comparing native and rhTF-activated TEG found that the addition of TF decreased reaction time (R) and clotting rate (K) and increased the rate of clot formation (α angle), indicating a procoagulant effect of adding TF. TF-TEG had a narrower standard deviation for R, K, α angle, and LY60 compared with native TEG, and interoperator differences were reduced. In another study, in healthy horses, the results indicated that horses had a tracing similar to those of other species, but the intrinsic and extrinsic pathways were less and more efficient, respectively.

The effect of time from sampling to analysis has been examined in both dogs and horses. The studies showed that canine citrated whole blood can be used for TEG analysis with diluted rhTF as activator when stored at room temperature for either 30 or 120 minutes. There was a statistically significant tendency toward hypercoagulability after 120 minutes compared with 30 minutes, however. Similar results were found in the study on horses. The results showed a significant effect of storage time on R, K, and α angle but not maximum amplitude (MA) after 30, 60 and 120 minutes of storage at room temperature. Both studies indicate that because there is incomplete inhibition of coagulation in citrated blood samples, a fixed time point should
be chosen for serial measurements in animals as is recommended in humans. Several studies have found that the pig is a suitable animal model for researching blood coagulation and fibrinolysis. Studies have therefore investigated the applicability of TEG in porcine blood, and normal values and reference intervals for porcine blood have been established for several different assays and compared with human reference intervals for the coagulation parameters investigated. Porcine blood has generally been found to be hypercoagulable irrespective of the type of assay or machine used. Except for the FibTEM and aPTT tests, all commercially available coagulation ROTEM tests seem to be fully applicable for porcine blood.21,22 Similarly, several studies have used TEG to study coagulation in pigs, both with native assays, with kaolin and diluted rhTF as activators.23–25 Interestingly, a study has indicated that TEG and ROTEM provide similar results in pigs, with a positive correlation between TEG and ROTEM for R, K, and MA parameters. Higher R/MA and lower α angle were seen in TEG samples than ROTEM samples; however, the magnitude of changes from baseline in hypercoagulable or hypocoagulable samples was equivalent with TEG and ROTEM, indicating comparable use of the instruments.26

Only one study attempted to examine species differences in the coagulation profile performed on ROTEM with blood from five different species: humans, rats, pigs, sheep, and rabbits. The clotting time was found to be comparable in humans and sheep both with and without thrombin stimulation. Interestingly, humans and sheep were 100-fold more sensitive to thrombin than rats, pigs, and rabbits. The maximum clot firmness with or without thrombin stimulation was similar in rabbits and humans. The maximum lysis with or without thrombin stimulation was similar in humans and pigs. Thus the authors conclude that sheep could be

### Table 1 Examples of Thromboelastographic Values in Various Selected Healthy Animal Species

<table>
<thead>
<tr>
<th>Specie</th>
<th>Assay</th>
<th>R (min)</th>
<th>K (min)</th>
<th>α Angle (degrees)</th>
<th>MA (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td>Native</td>
<td>1.7–8.2</td>
<td>1.3–4.5</td>
<td>46.3–71.4</td>
<td>43.5–61.0</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>1.8–8.6</td>
<td>1.3–5.7</td>
<td>36.9–74.6</td>
<td>42.9–67.9</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>2.8–8.7</td>
<td>2.3–7.7</td>
<td>27.5–58.7</td>
<td>39.0–59.0</td>
</tr>
<tr>
<td>Feline</td>
<td>Native</td>
<td>1.5–4.4</td>
<td>1.0–2.8</td>
<td>59.2–79.8</td>
<td>46.0–69.2</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>2.4–9.5</td>
<td>1.2–3.9</td>
<td>45.5–73.5</td>
<td>46.8–66.1</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>3.2–12.5</td>
<td>1.9–5.8</td>
<td>34.1–64.3</td>
<td>40.3–62.8</td>
</tr>
<tr>
<td>Rat</td>
<td>Native</td>
<td>1.37–5.49</td>
<td>NA</td>
<td>NA</td>
<td>69.7–78.5</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>1.0–3.4</td>
<td>0.3–1.1</td>
<td>77.8–86.2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Native</td>
<td>4.8–7.2</td>
<td>1.3–1.7</td>
<td>ND</td>
<td>64.4–69.2</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>0.9–2.1</td>
<td>0.6–1.0</td>
<td>80.2–84.2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Native</td>
<td>3.0–25.0</td>
<td>0.8–4.2</td>
<td>40.1–80.1</td>
<td>44.0–82.4</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>3.7–4.6</td>
<td>1.2–1.6</td>
<td>65.5–75.1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Equine</td>
<td>Native</td>
<td>11.0–23.0</td>
<td>3.2–8.4</td>
<td>14–70</td>
<td>48.9–71.7</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>3.8–9.4</td>
<td>1.1–5.1</td>
<td>32.9–68.9</td>
<td>52.1–72.4</td>
</tr>
<tr>
<td>Pig</td>
<td>Native</td>
<td>2.5–6.5</td>
<td>0.8–1.6</td>
<td>69.1–81.1</td>
<td>65.6–74.0</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>2.2–3.0</td>
<td>0.8–0.8</td>
<td>64.8–81.6</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Range.
1Mean ± 2 standard deviations.
2Mean ± 2 standard errors.
3Previously unpublished data from author.
R, reaction time; K, clotting rate; α angle, rate of clot formation; MA, maximum amplitude; TF, tissue factor; NA, not available; ND, no data.
Biological Variation

Every test result is subject to several sources of biological variation causing measurements in the same individual to change with time. Knowledge of these temporal changes is useful when establishing whether the performance of an assay is appropriate for interpreting results and making a diagnosis. Desired analytical imprecision and within-subject biological variation are particularly important here and the data can be used to (1) estimate the critical difference for significance between serial results, (2) assess the utility of conventional population-based reference ranges, and (3) set objective analytical performance standards. A study in dogs has investigated the biological variation of aPTT, PT, thrombin time, fibrinogen, antithrombin (AT), and D-dimer and compared it with four TEG parameters (R, K, α angle, and MA) on citrated canine plasma from clinically healthy dogs. The results showed that all the coagulation assays, fibrinogen, AT, and D-dimer showed a degree of individuality, which makes the use of population-based reference ranges alone an insensitive interpretation criterion, whereas a population-based reference interval seems to be sensitive for interpreting all TEG parameters. Analytical performance standards for imprecision were only met for one of the coagulation assays, whereas all TEG parameters except α achieved this analytical goal. Another study has looked at possible differences between sexes in dogs. The study did not find any difference in kaolin-activated TEG values between males and females. The difference in clotting activity between immature animals and adults has been examined in sheep and pigs. For example, fetal and neonatal lamb hemostasis has been studied from the 60th day of pregnancy to birth. Platelet counts and blood coagulation, as assessed by tests such as recalcification time and TEG, were similar in fetuses, neonates, and adult sheep. Finally, the effect of exercise on TEG results in horses has been examined and showed that reference intervals in racing horses, especially after exercise, were different from those of saddle horses, most likely due to a higher red blood cell (RBC) mass.

Blood Constituents Effect on Thromboelastographic Measurements

The contribution of platelets and soluble clotting components to clot strength has been the focus of several clinical studies using TEG in humans. From an experimental point of view it is relevant to know that the same correlations can be found in animals and this has been examined in several animal studies. In a study in rabbits, the contribution of platelet function and soluble components of the coagulation pathway to total clot strength were found to be similar to those in humans. It was found that G significantly correlates with platelet concentration in rabbits and thus the contribution of platelets to the total G is similar to that observed in humans. In a study on the effect of erythrocyte concentration on TEG parameters in mice, it was observed that increasing erythrocyte concentrations profoundly and reversibly inhibited thrombus formation and prolonged the time of clot development, most likely due to mechanical interference of RBC with clot-forming platelets. Similar results have been found in a study in dogs where native TEG values in greyhounds were compared with a group composed of other breeds. The results showed that greyhounds had significantly different values. They were more hypocoagulable, likely because of their high hematocrit. Clinical studies have shown that dogs with clinical signs of bleeding and are also hypocoagulable had decreased platelet count, prolonged PT, and increased D-dimer compared with normocoagulable dogs. The same study found that dogs that were hypercoagulable had a significant increase in fibrinogen compared with the dogs with normal TEG. Another study in dogs with disseminated intravascular coagulation (DIC) showed that when stratifying for hypo-, normo- or hypercoagulable TEG results based on MA, there were statistically significant differences with higher platelet count, fibrinogen, and plasminogen in hypercoagulable dogs, indicating that MA partially depends on these factors in dogs as in the other examined species. A similar recent study in dogs admitted to the intensive care unit (ICU) looked at the correlation of TEG results to other parameters and found hypercoagulable dogs had significantly increased D-dimer and fibrinogen concentrations compared with normocoagulable dogs. Significant correlations were found between MA and fibrinogen and between R and PT. One study in horses found that regression analysis indicated a significant positive relationship between MA and fibrinogen concentrations and R time and PT. These studies all indicate that the influence of blood components on TEG parameters is conserved across species.

APPLICATION

TEG has traditionally been used to monitor hemostatic function during surgery and to optimize blood product selection and use in experimental animals, but the role of TEG is now expanding to also include diagnosis and treatment of both hypocoagulable and hypercoagulable states. The use of TEG as a transfusion guide seems to have a large and thus far unused potential in clinical veterinary medicine. It is intuitively easy to interpret and thus represents a welcome potential to significantly
optimize blood product usage and efficacy in veterinary medicine. However, to date no studies have been published regarding the use of TEG to guide transfusion. TEG also has the potential to test the efficacy of different types of medication in vivo and in vitro, but perhaps the unique ability to identify hypercoagulability is even more important. TEG is exceptional in its ability to detect this possibly thrombotic state, and several studies have attempted to determine the cause of the hypercoagulability in individual disease states and the incidence of thrombosis in hypercoagulable animals; however, no one has yet been able to predict thrombosis before it occurs, and longitudinal studies will be needed to determine if TEG-identified hypercoagulability is sufficient as a risk factor to become a clinically valuable predictor of thrombosis and a target for therapy and preventive measures.

**CLINICAL STUDIES**

Compared with the amount of experimental data, the clinical data on the use of TEG in animals is still limited. TEG was first clinically employed in a study where it was used to detect hypercoagulability in dogs with parvovirus infection. Since then TEG has been used in a few clinical veterinary studies. So far the studies have mainly included dogs and horses, but from personal communications and data presented at international conferences, we are aware that several other species have been and are being studied at academic institutions, including clinical studies within critical illness, endocrinology, cardiology, and oncology. Preliminary data from these studies have been presented at conferences, but the publications have not yet been finalized and therefore they are not included in this review.

**Hypercoagulability and Thrombosis**

**ONCOLOGY**

In a recent study where TF-TEG was used to examine the overall hemostatic state in dogs with a variety of neoplasias, a total of 28 of 49 dogs (57%) had abnormal TEG results. Overall, a total of 22 dogs could be characterized as hypercoagulable (45%) and 6 were characterized as hypocoagulable (12%). Interestingly all the hypercoagulable dogs had malignant cancer with distant metastases. The results confirm that most dogs with a malignancy have hemostatic dysfunction, like humans, but most importantly, it documented for the first time that the most common abnormality is hypercoagulability.

**CRITICAL ILLNESS**

As in humans, many of the underlying conditions in dogs admitted to an ICU can cause hemostatic dysfunction. Several clinical studies using TEG have been performed on critically ill animals, primarily dogs. The first of these studies was on the use of TEG to diagnose hypercoagulability in a small number of dogs with hemorrhagic gastroenteritis due to canine parvovirus (CPV) infection. All dogs with CPV had evidence of hypercoagulability, determined on the basis of significantly increased native TEG MA. Interestingly, four of nine dogs with CPV had clinical evidence of venous thrombosis or phlebitis associated with catheters. The results of this first study proved that TEG could be used to detect hypercoagulability in dogs and that these hypercoagulable dogs had a high prevalence of clinical thrombosis, indicating a possible correlation between TEG-detected hypercoagulability and thrombosis.

In another study examining the application of rhTF-TEG as an aid in the diagnosis of DIC in dogs, it was demonstrated that TEG can be used to distinguish between different stages of DIC. Hemostatic dysfunction was observed in 33 of 50 of the dogs, of which 22 were hypercoagulable and 11 were hypocoagulable based on the clot strength (G value) alone. There were significant differences in K, α angle, and MA values among hypo-, normo-, and hypercoagulable dogs. Perhaps the most interesting finding in the study was a significant difference in case fatality rate between hypo- (64%) and hypercoagulable (32%) dogs (relative risk = 2.38). Thus the study was the first to show that TEG results could be used to predict outcome in critically ill dogs. The results indicate that TEG can be used to predict mortality, and the ability to stratify patients with DIC may potentially provide an option for more individually tailored treatment plans for patients with DIC in the near future. Interestingly a recent study in humans has demonstrated similar results. A very recent study screened dogs admitted to the ICU with TEG with the purpose of comparing TEG results and standard coagulation tests and investigating associations among the variables measured and found comparable prevalence and distribution of coagulopathies. Unfortunately neither of the studies looked at the incidence of thrombosis.

Immune-mediated hemolytic anemia (IMHA) in dogs is a disease associated with high mortality rates despite aggressive immunomodulatory treatment, and thrombosis has been documented at necropsy in up to 80% of dogs with IMHA. A recently published retrospective study used TEG to determine whether a hypercoagulable state was present in 39 dogs with IMHA. Thirty-three of 39 patients were hypercoagulable based on clotting index. The six remaining dogs were normocoagulable, and interestingly, these normocoagulable dogs had a significantly increased mortality rate compared with the hypercoagulable dogs.
Congenital Coagulopathies
A recent study examined the possible use of rhTF-activated and native TEG in the diagnosis of Scott’s syndrome (platelet procoagulant deficiency) in dogs. The results showed no significant differences between dogs with Scott’s and control dogs in any TEG parameter in TF-activated samples; however, nonactivated samples showed significantly prolonged mean K and time to maximal rate of thrombus generation in the dogs with Scott’s.\textsuperscript{39,40} ROTEM and TEG have both been used to assess the hemostatic potential in beagles with confirmed factor (F)VII deficiency, and the results revealed a severe impairment of clotting activity in affected beagles.\textsuperscript{39,40} In horses with clinical bleeding, TEG has been used to diagnose an 18-month-old Oldenbourg filly that presented with clinical bleeding. TEG was performed by using both kaolin and TF as activators. TEG reaction times were similar with kaolin and TF in the patient and a control horse. However, MA was decreased in the patient with both kaolin and TF. Platelet aggregation responses to adenosine diphosphate (ADP) and collagen were profoundly reduced in the affected horse. Flow cytometry showed an absence of CD41 and decreased expression of CD41/CD61-reacting antigen on the patient’s platelets, indicating a diagnosis of Glanzmann’s thrombasthenia.\textsuperscript{41}

Acquired Coagulopathies
The ability of a laboratory assay to reveal and correlate to clinical phenotype is crucial for rational hemostasis monitoring in clinical cases, but conventional coagulation assays often fail to show such correlation. A recent study demonstrated that rhTF-activated TEG is able to correctly identify dogs with clinical signs of bleeding with both higher positive and negative predictive values than a conventional coagulation profile consisting of aPTT, PT, D-dimer, fibrinogen, and platelet count, which are widely used in veterinary medicine.\textsuperscript{31}

EXPERIMENTAL STUDIES
Many animals are used in research on blood coagulation and fibrinolysis, but the relevance of animal models to human health is often questioned because of differences between species. The objective is mostly to find an appropriate animal species that mimics the relevant coagulation profile in humans most adequately.

Hypercoagulability and Thrombosis
ONCOLOGY
Hypercoagulability detected by TEG has been reported in carcinoma tumor growth in rats. One study looked at four groups: control, tumor-bearing rats, complete resection, and partial resection. The results of the study indicated a significant acceleration of coagulation in carcinoma tumor-bearing animals. Furthermore complete resection returned the coagulation status toward normal, whereas sham operation or a partial resection did not, indicating that, in this tumor model, hypercoagulability of the blood could be used to indicate the presence of residual tumor.\textsuperscript{42} These results have been substantiated in another study of carcinoma in New Zealand hares. The results showed that changes in the TEG values of native blood samples versus celite-activated samples could provide a qualitative assessment of the presence of carcinoma in the hares.\textsuperscript{43} A few studies have used TEG to monitor response to anticancer therapy. In one interesting study the anti-metastatic potential of new non-anticoagulant low molecular weight heparin (NA-LMWH) was compared with the LMWH enoxaparin in a melanoma mouse model. Only enoxaparin demonstrated a significant anti-coagulant effect; however, both NA-LMWH or enoxaparin reduced lung tumor formation by 70% and caused similar tissue factor pathway inhibitor release from endothelial cells.\textsuperscript{44}

MODELS OF CRITICAL ILLNESS
Validation of animal models of DIC designed to mimic human DIC are crucial to translate findings in research models to treatment modalities for DIC in humans. The International Society on Thrombosis and Haemostasis (ISTH) classifications of overt and nonovert human DIC have proven to have a high diagnostic accuracy, but the scoring systems have rarely been applied to animal models of DIC. A recent study used rabbit brain thromboplastin to induce DIC in a rabbit model and test the applicability of the ISTH criteria for a standardized diagnosis of DIC. The administration of thromboplastin induced a reproducible dose-dependent model of nonovert DIC according to the ISTH diagnostic criteria. A dose-dependent decrease in blood pressure, platelet count, and fibrinogen level was seen together with a dose-dependent increase in PT, aPTT, level of thrombin-antithrombin complexes, fibrin degradation products, and number of thrombi in lung vasculature. All dose groups showed a significant shortening of R and decrease in MA, likely due to a decrease in platelet count, after injection of TF. The clotting time then gradually normalized over time and returned to near baseline values after 90 minutes. This study shows that the nonovert ISTH score can be applied to evaluate severity and progression of DIC in a standardized manner in this thromboplastin-induced rabbit model.\textsuperscript{45} One of the classic, but rare, characteristics of DIC is a decrease in circulating fibrinogen. A study on rats with lipopolysaccharide (LPS) sepsis-induced DIC investigated the effect of fibrinogen concentrate on fibrinogen plasma levels and coagulation parameters assessed with
TEG. Following administration of LPS, thrombelasto- 
graphic measurements revealed a concurrent decrease in 
MA and an increase in reaction time. Treatment with 
fibrinogen concentrate resulted in a statistically signifi- 
cant dose-dependent amelioration of the measured co-
agulation abnormalities and a significant decrease in 
mortality. A study in pigs used ROTEM in a study to 
detect activation of coagulation in a pig model of 
traumatic brain injury. A dramatic shortening in time to 
clot initiation and an increase in clot propagation were 
observed after induction of intracranial hypertension as 
compared with the control group. These results were 
进一步 substantiated by a pronounced increase in throm-
bin generation and a significantly shortened PT in the 
intervention group. The results indicate that TF prob-
ably is the main trigger of hypercoagulopathy seen in 
connection to brain injury.47

Congenital Coagulopathies
TEG has been used in several studies to characterize 
the hemostatic potential in dogs with both FVII and 
FVIII deficiency. The heterogeneity among severe 
hemophilia A patients causes variable tendencies for 
bleeding that cannot be detected or predicted by routine 
coagulation tests. Exercise is an important component 
of overall hemophilia care; however, in patients with 
severe hemophilia, there is an increased risk of bleeding. 
TEG has been used in a study to assess the global 
hemostatic status of a group of severe hemophilic A 
dogs at rest and after a standardized period of exercise. 
The study demonstrated significant inter- and intra-
individual variations based on TEG patterns at rest and 
following acute exercise as well as significant improve-
ment of global hemostasis after exercise in most of the 
tested dogs. The study supports the use of TEG in the 
assessment of the hemostatic pattern in severe hemo-
philia A and provides a potential for using TEG 
evaluation in managing exercise regimens for hemo-
philia care.48 A study on the effects of a FVII mutation 
on coagulation in beagles determined the plasma coag-
ulation patterns among normal, carrier, and affected 
dogs using ROTEM. Six of 11 homozygous affected 
animals showed no clot even after 60 minutes. Quanti-
tative analysis of those that did form clots indicated that 
several ROTEM parameters were significantly affected 
in FVII-deficient dogs including clotting time, max-
imum velocity, and time to reach maximum velocity. 
There were no statistically significant differences in 
maximum clot firmness among normal, carrier, and 
affected dogs.49 A long-term study on the expression 
of cFVIIa adeno-associated virus resulted in a short-
ening of the PT, partial correction of the whole blood 
clotting time and TEG parameters, and a complete 
absence of spontaneous bleeding episodes in severely 
hemophilic dogs.49

Acquired Coagulopathies
There are few experimental reports on hemostatic 
changes during prolonged hypothermia. One study ex-
amined the hemostatic changes during mild to moderate 
hypothermia in dogs. Platelet count, platelet aggrega-
tion, and TEG were measured in each group. Results 
showed that platelet aggregation was significantly de-
creased and TEG coagulation time (R and K) was 
prolonged, indicating that long-term hypothermia in-
duces platelet dysfunction in dogs.50

Trauma-induced coagulopathy, acidosis, and hyp-
othermia form a “lethal triad” that is difficult to treat 
and associated with extremely high mortality in humans. 
The phenomenon has been studied in numerous animal 
models, some of which have used TEG to assess the 
extent of coagulopathy. In one such study on the 
coagulopathy of trauma, different coagulation tests 
were used to determine the best predictor of coagulo-
pathic bleeding and mortality in a small animal hemor-
hage model. Blood samples were analyzed by PT, 
apTT, TEG, and liver bleeding time (BT). Coagulopa-
thy increased BT, PT, and aPTT. TEG showed in-
creased reaction and clot formation times (R and K) and 
marked decrease in clotting rate (α angle and maximum 
velocity [Vmax]). Hemodilution hypothermia coagulopa-
thy, in contrast, increased only BT and aPTT and 
decreased the clotting rate (α angle and Vmax) and 
strength of the clot. The results indicate TEG measure-
ments of blood clotting rate are better indicators of 
coagulopathic bleeding and mortality in this lethal 
hemorrhage model than plasma-based clotting assays.51

Monitoring the Effect of Anticoagulant and 
Procoagulant Pharmacological Agents
These studies emphasize a unique feature of the TEG 
assay, which suggests it could be used in targeting 
therapy of the patient toward a normalization of the 
patient’s TEG tracings and hereby tailor dosage to meet 
the requirement of the individual patient.

For this application, TEG has been used to assess 
circulating heparin activity and the effect of antiplatelet 
and antifibrinolytic drugs. In the procoagulant treatment 
category, TEG has been used to monitor the effect of 
treatment with rFVIIa in rabbits.

Thus TEG has been used to evaluate the effects of 
unfractionated heparin, dalteparin, enoxaparin, or 0.9% 
saline in cats with cardiogenic arterial thromboembo-
lism. The results indicate that cats require higher dos-
ages and more frequent administration of LMWH to 
achieve human therapeutic anti-factor Xa activity of 0.5 
to 1 U/mL. However, the study examined the effect at 4 
hours post injection, whereas peak anti-Xa activity is 
predicted at 2 hours after administration of LMWH.52

A veterinary example of the effects of LMWH on dogs is 
an in vitro study where heparinase-modified TEG was
investigated as a possible method to evaluate the effect of therapy with dalteparin in dogs. The results showed that spiking citrated canine whole blood with increasing doses of dalteparin significantly and dose dependently affects all TF-activated TEG parameters. In contrast to this, it was observed that when using kaolin as an activator there was almost no measurable dalteparin effect. An example of in vivo anticoagulant effect of minidose heparin (80 IU/kg) on surgery has been evaluated in dogs. The study did not show any effect of minidose heparin on the hemostatic parameters as measured by TEG, and heparin did not prevent the accelerated coagulation associated with surgery. A study compared the sensitivity of TEG, aPTT, and activated coagulation time (ACT) values with changes in anti-Xa activity after small-dose heparin administration of 0, 10, 20, and 30 U/kg of intravenous heparin in rabbits. TEG variables (R and α) significantly changed between 0, 10, and 20 U/kg heparin doses, but a difference between 20 and 30 U/kg could not be discerned secondary to loss of a detectable clot. The aPTT was significantly different between 0, 20, and 30 U/kg doses. ACT values were significantly different between the 0 U/kg heparin dose and all other doses; however, there were no significant differences between the 10, 20, and 30 U/kg heparin doses. Changes in anti-Xa activity were significantly linearly related to R, α, aPTT, and ACT. The results of the study indicate that TEG more sensitively reflects changes in circulating heparin activity than aPTT and ACT after small-dose heparin.

TEG has also been used recently to monitor the antiplatelet effect of various nonsteroidal anti-inflammatory drugs and clopidogrel. Dogs received aspirin, carprofen, deracoxib, and meloxicam. The results showed that platelet aggregation decreased after treatment with only aspirin and carprofen. TEG obtained after treatment with carprofen revealed decreased MA and α angle, suggesting hypocoagulability, whereas MA and coagulation index increased after treatment with deracoxib. A study has examined the in vitro effect of the glycoprotein IIb/IIIa antagonist tirofiban on porcine blood platelets. The obtained results reveal that in porcine platelets, the maximal concentrations of tirofiban used in human medicine (250 ng/mL) effectively block platelet responses triggered by ADP, partly block those induced by collagen, and very poorly block those evoked by thrombin; however, TEG measurements indicated that tirofiban, up to concentrations of 2000 ng/mL, did not affect the kinetics of TF-induced clot formation. The effect of antifibrinolytics has been studied several times in dogs with the use of TEG. It has also been used to examine the effect of tissue plasminogen activator (tPA) in pigs. Results showed the clots formed from porcine whole blood to be highly resistant to tPA-catalyzed lysis, and authors hypothesize that the resistance of porcine plasminogen to activation by tPA could be the explanation and further suggest caution in using the pig as an experimental model when studying the effects of various agents on fibrinolysis. This finding is in contrast to a 2008 study that showed the pig may be a good translational model for studying fibrinolysis.

To test the efficacy of rFVIIa in thrombocytopenia, a preclinical study was conducted in thrombocytopenic rabbits. Administration of rFVIIa significantly shortened the prolonged BT in thrombocytopenic animals as well as significantly reducing the blood loss. This effect was also reflected by a significant reduction of the PT, aPTT, as well as improvement in clotting parameters in an in vitro TEG thrombocytopenia model. Histopathological evaluation of kidney biopsies for the presence of micro thrombi did not reveal evidence of prothrombotic effects of rFVIIa in this model.

**CONCLUSION**

TEG has been used for many years in veterinary medicine, especially for experimental animal studies. In the last decade, however, the clinical application of TEG has increased significantly in the same areas as used in human medicine. There is still a vast potential for further clinical research, especially in using TEG to guide interventional therapy such as transfusions and procoagulant and antithrombotic treatment in relevant patient groups. The latter will be particularly interesting to follow in the future as more information emerges regarding the possible connection among various types of hypercoagulability detected with TEG and the development of thrombosis.

**REFERENCES**

7. Franco RF, de Jonge E, Dekkers PE, et al. The in vivo kinetics of tissue factor messenger RNA expression during
39. Poller L, Thomson JM, Sear CH, Thomas W. Identification of a congenital defect of factor VII in a colony of beagle dogs: