Preanalytic errors attributable to collection of blood samples should be avoided, especially when evaluation of the activation of the coagulation process is of interest. The optimum technique for collection of blood samples for use in coagulation analysis has been widely accepted as minimally invasive venipuncture, which has been considered to minimize activation of the coagulation cascade and provide the most accurate measurements.1 A study2 in dogs revealed that measurements of PT, aPTT, antithrombin activity, and protein C concentration did not differ significantly between venous and arterial blood samples; however, differences in fibrinogen concentration and thrombin time were detected. For venous samples, acceptable agreement between measurements of PT, aPTT, and fibrinogen concentration collected via an indwelling IV catheter and direct venipuncture was detected in dogs.3,4

Only a limited number of variables that reflect coagulation activity have been evaluated in these aforementioned investigations. Furthermore, the diameter of the needle used for sample collection was not taken into account. In humans, the effect of sample collection techniques on coagulation has been investigated. There is evidence that an increase in shear stress may activate coagulation via increased shedding of procoagulant-containing platelet microparticles.5,6

The influence of sample acquisition on coagulation variables is important for personnel involved in clinical and experimental settings. Therefore, the objective of the study reported here was to investigate the influence of sample collection technique on kaolin-activated TEG variables determined with recalcified blood, platelet function assessed via impedance-based aggregometry, secondary hemostasis (PT, aPTT, fibrinogen concentration, and activity of factor VIII), fibrinolysis (fibrin

**Objective**—To investigate the influence of diameter of the catheter and blood collection technique on platelet function and variables reflecting secondary hemostasis, physiologic anticoagulation, and fibrinolysis in dogs.

**Animals**—6 healthy Beagles.

**Procedures**—Blood samples were collected with 20- and 18-gauge venous catheters immediately after catheters were inserted in a peripheral vein, through a 14-gauge central venous catheter that had been placed via the Seldinger technique in a jugular vein <30 minutes before sample collection, and through a 13-gauge central venous catheter placed via a catheter-through-the-needle technique <30 minutes before sample collection (techniques 1 to 4, respectively). Platelet function was assessed in hirudin-anticoagulated whole blood via an impedance-based aggregometer with collagen (0.8, 0.4, 0.2, 0.1, and 0.05 µg/mL) as an inductor. Kaolin-activated thromboelastography variables were determined in citrated whole blood. Prothrombin time, activated partial thromboplastin time, fibrinogen and fibrin D-dimer concentrations, and activity of factor VIII, antithrombin, protein C, and protein S were assessed automatically in citrated plasma.

**Results**—At 0.05 µg of collagen/mL, the highest median rate of aggregation was observed for collection techniques 2 and 3 with 4.3 (range, 2.5 to 6.5) and 3.7 (range, 2.8 to 8.3) aggregation units/min; however, these values were not significantly different from values for the other collection techniques. Generally, sample collection technique did not have a significant impact on results of coagulation variables investigated.

**Conclusions and Clinical Relevance**—Various blood collection techniques can be used to obtain samples for coagulation testing. (Am J Vet Res 2011;72:64–72)
D-dimer concentration), and physiologic anticoagulants (activities of antithrombin, protein C, and protein S). Our hypothesis was that collection of a sample through catheters inserted via the Seldinger technique or through needles with a small diameter would result in activation of coagulation.

**Materials and Methods**

**Animals**—Six healthy Beagles (4 neutered males and 2 spayed females) that were 2 to 3 years old (median age, 2.5 years) were used in the study. Dogs were assessed as healthy on the basis of results of physical examination, a CBC, renal function evaluation (urea and creatinine concentrations), and hepatic function evaluation (total bilirubin concentration and activities of alkaline phosphatase, alanine aminotransferase, and glutamate dehydrogenase) and measurement of electrolytes (sodium, potassium, ionized calcium, magnesium, and phosphate) and plasma concentrations of total protein, albumin, globulin, and fructosamine. The investigation was approved by the Ethics Committee for Animal Welfare, regional board, Giessen, Germany.

**Study design**—In each dog, the same assessments were conducted on blood samples collected by each of 4 techniques to obtain an intraindividual and interindividual comparative analysis of the sample collection techniques. Platelet function was assessed with an impedance-based whole blood platelet function analyzer. Variables characterizing secondary hemostasis, physiologic anticoagulants, and markers of fibrinolysis were determined with an automated coagulation analyzer. Assays conducted with this analyzer included PT, aPTT, measurement of plasma concentrations of fibrinogen and D-dimer, and measurement of activities of factor VIII, antithrombin, protein C, and protein S. In addition, a kaolin-activated TEG analysis was performed.

**Sample collection**—Blood samples were obtained within a 2-hour period from resting dogs from which food had been withheld for the preceding 24 hours. Samples were obtained with a 20-gauge intradermic needle in a cephalic vein (technique 1); an 18-gauge, 45-mm venous catheter placed in the contralateral cephalic vein (technique 2), a central venous indwelling catheter (14-gauge, 16-cm radiopaque polyurethane catheter) inserted in a jugular vein via the Seldinger technique (technique 3), and a central venous indwelling catheter (13-gauge polyurethane catheter inserted in the contralateral jugular vein via a catheter-through-the-needle method described elsewhere; technique 4). Each dog served as its own control animal. Order of sample collection was via the 18-gauge venous catheter, the 20-gauge intradermic needle, the 14-gauge central venous catheter inserted by use of the Seldinger technique, and the 13-gauge central venous catheter inserted by use of the catheter-through-the-needle technique (ie, techniques 2, 1, 3, and 4, respectively). For techniques 1 and 2, sample acquisition was performed immediately after insertion of the needle and catheter, respectively, and blood was allowed to flow directly into anticoagulant-containing tubes. For techniques 3 and 4, samples were acquired within 30 minutes after insertion of the central venous catheters, and the specimens were carefully aspirated into a plain 5-mL syringe and then gently placed into anticoagulant-containing tubes. When sample collection was not performed immediately after insertion of a central venous catheter, the catheter was flushed with saline (0.9% NaCl) solution and the first 4 to 5 mL of aspirated blood was discarded. Blood samples were collected into a 1.2-mL EDTA tube, a 10-mL silicone-lined evacuated tube containing 3.13% sodium citrate, a 1.2-mL tube containing 3.18% sodium citrate, and a 4.5-mL tube containing the thrombin inhibitor hirudin at a concentration of 25 μg/mL.

The EDTA blood sample was used for platelet counts. The citrate tubes were carefully inverted several times to ensure mixing of citrate and blood (9 parts blood:1 part citrate). Samples were carefully examined to ensure tubes were filled properly, and only samples with an exact ratio of 9 parts blood:1 part citrate were included. The 1.2-mL citrated tubes were stored at 22°C for 60 minutes before subsequent TEG analysis. The 10-mL evacuated tubes containing sodium citrate—whole blood were used for characterization of secondary and tertiary hemostasis and were centrifuged (850 X g for 10 minutes) within 1 hour after sample collection. Citrated plasma was separated from the erythrocytes and centrifuged again (850 X g for 10 minutes) to remove all nonsedimented platelets, as recommended elsewhere. The supernatant was removed and stored at −80°C until analysis. Analysis was performed within 3 weeks after sample collection. For all analytes, sample stability was > 12 months. Immediately before analysis, plasma samples were thawed at 37°C in a water bath, as recommended elsewhere, to ensure the cryoprecipitate completely dissolved. Samples then were centrifuged (850 X g for 10 minutes) to ensure that the plasma was mixed.

Hirudin-anticoagulated blood was used for whole blood aggregometry. Platelets were allowed to sit undisturbed for 30 minutes at 22°C prior to analysis.

**Test methods for secondary hemostasis, fibrinolysis, and natural anticoagulants**—Test validation and establishment of reference intervals for coagulation variables evaluated by use of the automated coagulation analyzer have been reported elsewhere. Coagulation screening tests (PT and aPTT) were performed automatically by use of commercial reagents. Fibrinogen concentration was determined via the Clauss method by use of a human plasma calibration standard provided by the manufacturer.

Fibrin D-dimer concentration was measured with an automated assay as described elsewhere. Antithrombin activity was detected on the basis of inhibition of thrombin with a chromogenic substrate kit. Antithrombin activity was reported as a percentage of the value determined for the human plasma calibration standard.

For measurement of protein C activity, an automated functional clotting test was performed in accordance with the manufacturer’s instructions. Each patient sample was diluted 1:5 with diluent buffer. Results were reported as the percentage of the value determined for the canine pooled plasma calibration standard. The canine pooled plasma calibration stan-
standard was prepared from citrated plasma obtained from 16 healthy adult (median age, 3.5 years) dogs and was used for all coagulation tests (ie, protein C, protein S, and factor VIII) that required a comparison with results for the canine pooled plasma calibration standard.

Protein S activity was also determined with a functional automated clotting test. Each patient sample was diluted 1:5 with diluent buffer. Results were reported as the percentage of the value for the canine pooled plasma calibration standard.

Measurement of factor VIII was performed via a modified 1-stage aPTT assay with human factor VIII-deficient substrate plasma. Each patient sample was diluted 1:40 with diluent buffer. In the case of samples with factor VIII activity > 150%, measurement was automatically repeated at a dilution of 1:60. Results were reported as the percentage of the value for the canine plasma pool calibration standard.

Two internal quality control materials provided by the manufacturer were included in each measurement for most of the variables, including PT, aPTT, fibrinogen, antithrombin, and fibrin D-dimer. A third quality control material was available and was included for PT, aPTT, fibrinogen, and antithrombin measurement.

For protein C, protein S, and factor VIII activities, which were reported as the percentage of the activity for the canine pooled plasma (ie, compared with a standard curve created by use of the canine pooled plasma),

Figure 1—Box-and-whiskers diagrams depicting results for variables that reflect secondary hemostasis, physiologic inhibitors of coagulation, and fibrinolysis (PT [A], aPTT [B], fibrinogen concentration [C], protein S activity [D], protein C activity [E], factor VIII activity [F], antithrombin activity [G], and fibrin D-dimer concentration [H]) for blood samples collected from 6 dogs by use of 4 collection techniques (technique 1, 20-gauge intradermic needle in a cephalic vein; technique 2, 18-gauge, 45-mm venous catheter placed in the contralateral cephalic vein; technique 3, central venous indwelling catheter [14-gauge, 16-cm radiopaque polyurethane catheter] inserted in a jugular vein via the Seldinger technique, and technique 4, central venous indwelling catheter [13-gauge polyurethane catheter] inserted in the contralateral jugular vein via a catheter-through-the-needle method). For each technique, the box represents the values from the first to third quartile (25th to 75th percentile). The horizontal line in each box represents the median. The whiskers represent the range; values outside (less than the lower quartile minus 1.5 times the interquartile range or greater than the upper quartile plus 1.5 times the interquartile range) or far outside (less than the lower quartile minus 3 times the interquartile range or greater than the upper quartile plus 3 times the interquartile range) the range are indicated as black circles and black squares, respectively. The dotted lines indicate the reference interval. Results for activities of protein C, protein S, and factor VIII represent a percentage of the value determined for a canine pooled plasma calibration standard. Results for antithrombin activity represent a percentage of the value determined for a human plasma calibration standard. Significance was set at values of \( P < 0.002 \) after Bonferroni correction. Values did not differ significantly among collection techniques for any of the variables (PT \( [P = 0.197] \), aPTT \( [P = 0.375] \), fibrinogen concentration \( [P = 0.375] \), protein S activity \( [P = 0.230] \), protein C activity \( [P = 0.088] \), factor VIII activity \( [P = 0.431] \), antithrombin activity \( [P = 0.431] \), or fibrin D-dimer concentration \( [P = 0.181] \).
internal quality control was performed with citrated plasma obtained from a healthy Beagle. Undiluted citrated plasma was used as a normal control sample. The abnormal control sample was prepared by diluting the normal control citrated plasma with saline solution (dilution, 1:1). Normal and abnormal control samples were stored in aliquots of 0.5 mL at –80°C until analysis.

Interassay CV obtained from 15 to 20 measurements ranged between 5% and 10% for PT and aPTT and between 10% and 16% for fibrinogen concentration, antithrombin activity, protein C activity, and protein S activity. The interassay CV for D-dimer concentration in abnormal control samples (D-dimer concentration, 2.4 µg/mL) was between 10% and 16%, whereas the interassay CV for D-dimer concentration in normal control samples was 16.7%. For factor VIII, interassay CV was 24.3% to 28.9%.

TEG—Test validation and establishment of reference intervals for coagulation variables for kaolin-activated TEG have been reported previously.11 The TEG analysis of recalcified citrated whole blood was performed as described elsewhere11 in accordance with the manufacturer’s recommendations by use of 2 TEG analyzers and thus 4 TEG channels. Two internal quality control materials (normal and abnormal) were assayed each day of analysis. In addition, an electrical internal quality control test (ie, e-test) was performed.

The TEG variables evaluated in the study were interpreted as reported elsewhere.11–16 Briefly, R is the reaction time, which is a measure of clot initiation that mainly reflects secondary hemostasis, and K is the coagulation time needed until tracings reach an amplitude of 20 mm. Angle α indicates the rapidity of fibrin cross-linking, and MA is a measure of clot strength. For the computerized TEG, G is a measure of clot rigidity and thus the overall coagulation state and is calculated by use of the following equation:17

\[ G = \frac{5,000 \times MA}{96 – MA} \]

Whole blood aggregometry—To assess platelet function, whole blood aggregometry was performed by use of an impedance-based platelet function analyzer. Aggregometry was performed automatically with single-use test cells6 with 2 incorporated sensor units; each of the incorporated sensor units had 2 metal electrodes. For induction of platelet aggregation, collagen7 was used at 5 final concentrations (0.8, 0.4, 0.2, 0.1, and 0.05 µg/mL).

Figure 2—Box-and-whiskers diagrams depicting results for the TEG variables R (A), angle α (B), MA (C), G (D), and K (E) obtained for blood samples collected from 6 dogs by use of various techniques. Values did not differ significantly among collection techniques for any of the variables (R [P = 0.237], angle α [P = 0.230], MA [P = 0.194], G [P = 0.760], or K [P = 0.431]). See Figure 1 for remainder of key.

Figure 3—Median and range for platelet aggregation (A) and rate of aggregation (B), which were assessed with a whole blood impedance-based aggregometer on samples obtained from 6 dogs by use of 4 blood collection techniques (technique 1, stippled bars; technique 2, white bars; technique 3, dark gray bars; and technique 4, horizontal-striped bars). Collagen at 5 final concentrations was used as an inductor. Significance was set at values of \( P < 0.002 \) after Bonferroni correction. Effect of sample collection technique and the interaction between collection technique and collagen concentration did not significantly affect platelet aggregation (\( P = 0.065 \) and \( P = 0.746 \), respectively) or rate of aggregation (\( P = 0.048 \) and \( P = 0.729 \), respectively); however, collagen concentration significantly affected both platelet aggregation (\( P < 0.001 \)) and rate of aggregation (\( P < 0.001 \)). AU = Aggregation units. See Figure 1 for remainder of key.
Aggregation was performed with hirudin-anticoagulated whole blood in accordance with the manufacturer’s instructions. Activated platelet function was recorded for 20 minutes. Measurements were always performed in duplicate; the mean was calculated automatically by the computer software and was used for statistical analysis. An electrical internal quality control test (electronic control test) was performed once each day.

**Statistical analysis—**Results were depicted as box-and-whisker plots. For TEG variables and variables reflecting secondary and tertiary hemostasis, laboratory-specific 95% reference intervals were available, which had been calculated from results obtained for 56 healthy adult dogs as described elsewhere. Sample acquisition for establishment of reference intervals was consistent with technique 2 in the present study.

Differences between results obtained for the 4 sample collection techniques were assessed with a Friedman test by use of commercial statistical software. A nonparametric test was chosen for all analyses because it is virtually impossible to prove a normal distribution with a sample size < 12. The impact of sample collection technique on whole blood aggregometry was assessed; data were logarithmically transformed and analyzed via an ANOVA and an ANCOVA by use of statistical software. Covariables were the collagen concentration as well as the interaction between sample collection technique and collagen concentration. Significance for all analyses was set at values of $P < 0.002$ after Bonferroni correction.

**Results**

Sample collection technique did not significantly influence any variable reflecting primary, secondary, or tertiary hemostasis or natural inhibitors of coagulation (Figures 1–3). There was a slightly higher (but not significantly so) median TEG G value and median area under the aggregation curve at a low inductor concentration of 0.05 µg of collagen/mL, which reflected platelet function was evident in samples collected through venous catheters inserted via the Seldinger technique.

At the lowest collagen concentration of 0.05 µg/mL, the highest median aggregation rate was detected for collection techniques 2 and 3. However, after Bonferroni correction, highest median aggregation rate did not differ significantly among the collection techniques.

The 2-way test of variance revealed a significant influence of the inductor (collagen) concentration on the area under the aggregation curve and rate of aggregation (Figure 3).

Despite the lack of significant differences among the groups, protein S activity was lower in samples obtained via collection technique 3. Results obtained with samples obtained via collection technique 2 appeared to correspond best with the reference intervals (Figures 1 and 2). High variability among the collection techniques was detected for fibrin D-dimer concentration and activities of factor VIII, protein C, and protein S.

One female dog had mild to moderate deficiency of factor VIII that was detected in samples obtained via all 4 collection techniques. In another dog, mild to moderate hyperfibrinogenemia was detected in samples obtained via all 4 techniques of sample acquisition (Figure 1).

**Discussion**

Analysis of the results reported here clearly indicated that sample collection technique did not have a significant impact on primary, secondary, and tertiary hemostasis or the inhibitors of coagulation. In the present study, platelet function was increased (but not significantly so) after sample acquisition through catheters inserted via the Seldinger technique.

Platelet activation and thrombus formation have been intensively studied in domestic animals used in experiments as well as in people undergoing coronary catheter interventions. In sheep, catheter guide wires caused histologically detectable endothelial cell damage, platelet aggregation, and thrombus formation, which was higher when the guide wire was manipulated manually. Therefore, it can be hypothesized that insertion of the central venous catheter via the Seldinger technique by use of a guide wire may have induced a higher degree of endothelial cell damage and subsequently platelet activation than for the over-the-needle technique. Moreover, blood collected from humans through catheters introduced with a guide wire has much higher plasma heparin-neutralizing activity, platelet factor 4 concentration, and β thromboglobulin concentration than does peripheral blood. Electron microscopic evaluation of tips of guide wires used during routine angioplasty procedures in humans has revealed a thrombus formation rate of 26% to 80%, depending on the type of guide wire. In a static experiment with human whole blood, excessive thrombus formation was detected for heparin-free guide wires, whereas guide wires impregnated with sodium heparin remained essentially free of thrombi. However, these examples represent fairly aggressive intravascular manipulations, compared with the generally mild and largely nontraumatic insertion of the guide wire of a catheter via the Seldinger technique; therefore, the results of the aforementioned studies are hardly comparable to those obtained for the experiments reported here.

Investigations in humans have revealed that a reduction of the vascular lumen may result in an increase in shear stress and thus platelet activation. However, shear stress of platelets induced by a small diameter of the needle or catheter was unlikely to be of importance in the study reported here because the highest activation of platelets would have been expected in samples collected via direct venipuncture with a 20-gauge needle or through an 18-gauge catheter. For the central venous catheters, blood had to be aspirated with a syringe, which may have contributed to platelet activation in the present study. In an experimental investigation with samples of human whole blood, it was found that shear stress (ie, an induced tangential blood force that primarily depends on the lumen diameter of the needle) and also the shear rate may induce platelet activation and formation of platelet microparticles. In the present study, there was a lower shear rate when samples were collected through a needle or a venous
catheter inserted in a small peripheral vein because blood was allowed to flow directly into a citrated tube, which was in contrast to sample acquisition through venous catheters whereby blood was slowly aspirated with a syringe and then placed into tubes.

Regarding secondary hemostasis, the results reported here are in accordance with those in another study in 14 healthy dogs in which investigators detected no significant difference in PT, aPTT, fibrinogen concentration, and fibrin degradation products in samples obtained via 16-gauge catheters inserted in a jugular vein or via venipuncture (diameter of the needle was not specified). In contrast to the present study, catheters in that other study were heparinized before insertion in a jugular vein; however, a 2-syringe method was used for sample collection and the first volume of blood was discarded to avoid any effect of heparin on coagulation variables. Comparable results were also obtained for 120 human patients receiving anticoagulation treatments, with statistically equivalent measurements of PT and aPTT when samples were collected through a 21-gauge needle (venipuncture method) or 18-, 20-, or 22-gauge peripheral venous catheters (peripheral venous catheter method).

A similar outcome was reported for aPTT, PT, and factor VIII activity in 6 patients with hemophilia A under nonbleeding conditions when comparing samples obtained via peripheral venipuncture with samples obtained through a venous catheter.

In contrast to results of the present study and other findings in dogs and humans, results of a recent study in dogs hospitalized in an intensive care unit revealed that the aPTT determined in blood samples collected via venipuncture (reference group) with a 21-gauge needle was significantly shorter than the aPTT for samples obtained by use of a 20- or 18-gauge catheter (experimental group) when the samples were collected on the first day of catheter insertion. Despite the significant differences, the difference between aPTT results determined for the reference and experimental groups was small and therefore not considered clinically relevant.

Moreover, use of an even smaller 22-gauge catheter did not have a significant impact on aPTT in that study.

To the authors’ knowledge, there is no information in the literature regarding the effect of sample collection technique on protein S activity. The exposure of foreign surfaces (ie, a catheter) is followed within seconds by nonspecific protein adsorption, which can initiate blood coagulation. Therefore, it can be hypothesized that an initiation of thrombus formation may be followed by a counter-regulation and thus the activation and subsequent consumption of protein C and protein S. However, in that case, an equal decrease of protein C and protein S activities would be expected, which was not detected in the study reported here.

It was our objective for the present study to investigate the sole effect of sample collection technique on coagulation variables; thus, heparinization of the catheters was strictly avoided. However, the influence of sample collection via a heparinized central venous catheter has been investigated in humans and published as an integrative literature review. Based on the reviewed studies, the authors reported that a significant prolongation of aPTT could be detected when blood samples were collected directly from the catheter, which was not evident when > 6 volumes of the catheter lumen were discarded, as was reported in an integrative retrospective study in humans.

Regarding the methods used in the study reported here, a novel impedance-based whole blood aggregometer was chosen to assess platelet function because of the fact that light transmission aggregometry (ie, the Born method, which is the most commonly used method for platelet function testing) has several disadvantages. Disadvantages of the Born method include the need for preparation of platelet-rich plasma, which results in separation of other blood cells from platelets and is known to influence platelet function, and the loss of platelets during the process of preparation (as determined in samples obtained from humans), and the fact that giant platelets (which may be hyporeactive or hyperactive) are generally not included in platelet-rich plasma.

The impedance aggregometer used in the present study determined platelet function in diluted whole blood by use of disposable test cells with duplicate impedance sensors. Thus, possible sources of error (eg, cleaning of electrodes between subsequent analyses) that had to be performed in older impedance aggregometers were avoided. In the present study, the thrombin antagonist hirudin was chosen as an anticoagulant, as previously recommended for dogs because it preserves the physiologic concentrations of ionized calcium and magnesium better than does citrate. In contrast to a previous investigation for dogs, much lower inductor concentrations for detection of a possible activation of platelets were chosen in the study reported here. However, it must be considered that lower inductor concentrations result in higher intra-assay variation.

For concentrations of fibrin D-dimer and activities of factor VIII, protein C, and protein S, a high variability was detected among the collection techniques. This was most likely attributable to a comparatively high intra-assay and interassay variation for these variables. Intra-assay CV for fibrin D-dimer was 45.9%; however, it must be considered that concentrations of fibrin D-dimer are low in healthy dogs, which is known to result in high intra-assay CVs. The 6-run intra-assay repeatability for fibrin D-dimer in another study was 6.1% (range, 2% to 10%) for the same method and analyzer when determined in canine plasma (n = 6 dogs) with increased fibrin D-dimer plasma concentrations that ranged from 0.94 to 3.08 μg/mL.

For most variables, results obtained for collection technique 2 best fit the respective reference intervals. This finding was not surprising because sample acquisition for establishment of the reference intervals was performed with technique 2.9,11 In the present study, kaolin was used as an inducer for TEG analysis. Other investigators performed TEG in canine samples without any inducer or with the activator TF. A study in humans indicated that R time in normal samples (control group) was shorter for kaolin-activated TEG than for TF-activated TEG. However, the direct comparison of kaolin- and TF-activated TEG in the control samples was not the objective.
that study, so the possibility that the difference was significant was not investigated. On the basis of the results of that study in humans, kaolin appears to be a stronger activator than does TF; thus, a mild activation of secondary hemostasis may have been masked by a strong activation of coagulation if the same situation applies to dogs. A more recent study in humans in which investigators compared the differences between kaolin- and TF-activated TEG revealed that activation with kaolin and TF in a dilution of 1:17,000 accelerated only the initiation phase of coagulation, which resulted in a shorter R time than for activation with TF in a dilution of 1:42,500. Thus, the authors of that study concluded that both activators can be used.

In the present study, kaolin was chosen as an activator because laboratory reference intervals have been established for this activator; thus, the results could be interpreted in relation to the reference interval. A second reason was the fact that many clinical studies use an activator of the coagulation process to simulate the in vivo process of coagulation. Tissue factor has been considered the best activator for this purpose, but TF is not available in ready-to-use vials and is thus less likely to be used routinely in a clinical laboratory. Moreover, TF has to be diluted from a stock solution, which results in a higher probability of preanalytic errors.

Because of its ease of use and the fact that reference intervals are available for dogs, kaolin-activated TEG is an attractive technique for clinicians. Although it cannot be ruled out that mild activation of secondary hemostasis attributable to sample collection may have been masked by strong activation of coagulation with kaolin-activated TEG, results of the present study clearly indicated that in a clinical setting, the type of sample collection technique did not influence TEG results when kaolin was used as an activator. In routine veterinary practice, anatomic location for sample acquisition or sample collection techniques often vary during follow-up examinations of a patient; therefore, kaolin-activated TEG can be recommended.

Regarding the preparation of citrated plasma, it must be mentioned that the centrifugation force routinely used in the authors’ laboratory was <1,500 × g (ie, the force recommended by the Clinical and Laboratory Standards Institute). However, according to recommendations made by the Clinical and Laboratory Standards Institute, alternate times and forces may be used providing that the plasma platelet count is ≤10 × 10⁹ cells/L, which can be achieved by use of the protocol we used in the study reported here. Even for human laboratories, a great degree of variation in centrifugation force (range, 300 to 3,000 × g) has been reported.

A limitation of the present study was the comparatively low number of dogs from which samples were obtained. Thus, significant effects may not have been detected.

Another limitation is the fact that samples were always collected in the same order, which could have influenced the results obtained (particularly the coagulation variables for the samples that were collected last). Theoretically, venous puncture and insertion of catheters may have induced activation of coagulation. In humans, problematic venipuncture may result in spurious activation of the hemostatic system; however, this appeared to be unlikely in the present study because no significant impact of the collection technique on the results was detected. Furthermore, only short-term effects were assessed in the study reported here, although it could be hypothesized that a catheter remaining in place for a longer period may activate the coagulation system. Therefore, it is possible that the results may not be representative for all clinical settings because most catheters used for collection of blood samples from patients will have been in place for >30 minutes and will have been heparinized. Further investigations to examine the effect of catheters remaining in place in a peripheral or jugular vein for a prolonged time period are warranted.

On the basis of results of the study reported here, it can be concluded that various blood collection techniques can be used to obtain samples for coagulation testing.


