Preanalytic factors, including blood collection site, technique, or materials, may affect the results of coagulation testing. Trauma to perivascular tissue, as with difficult venipuncture, leads to the release of tissue factor, which may initiate coagulation in vitro and result in shortening of activated clotting time, platelet activation or clumping, and clotted samples. Other factors include prolonged venous stasis, inappropriate filling and mixing of blood tubes, and variation within and between individuals processing the sample. Needle size is also a factor; for example, blood obtained with a 25-gauge needle has significantly higher D-dimer concentrations and lower platelet counts than does blood obtained with a 21-gauge needle.

The use of thromboelastography to evaluate hemostasis in dogs has increased in recent years. Thromboelastography has been used to characterize hemostatic changes in dogs with conditions such as parvoviral enteritis, immune-mediated hemolytic anemia, neoplasia, hypothermia, disseminated intravascular coagulation, and treatment with unfractionated heparin. The authors of these and other studies have used different techniques to obtain and handle blood samples prior to analysis. Several authors have reported collecting blood with a 21-gauge butterfly needle and an evacuated tube collection system. Others used evacuated tube collection systems with >1 type of needle, aspiration of blood from a CVC, or arterial catheter sampling, and a combination of methods.

Comparison of venous sampling methods for thromboelastography in clinically normal dogs

Julie M. Walker, DVM; Rita M. Hanel, DVM; Bernard D. Hansen, DVM, MS; Alison A. Motsinger-Reif, PhD

Objective—To evaluate effects of blood collection method and site on results of thromboelastography in healthy dogs.

Animals—8 clinically normal purpose-bred dogs.

Procedures—Blood was collected from the external jugular vein by syringe aspiration via direct venipuncture with a 20-gauge needle, through a central venous catheter, or into an evacuated tube with a 21-gauge winged needle catheter. Blood was collected from the lateral saphenous vein by syringe aspiration via direct venipuncture with a 20-gauge needle or into an evacuated tube with a 21-gauge winged needle catheter. Kaolin-activated thromboelastographic analyses were performed, and R (reaction time), K (clot formation time), α angle, maximal amplitude, and G (global clot strength) were analyzed.

Results—No significant differences were observed with regard to sampling site. Sample collection method had no effect on thromboelastographic results for saphenous vein samples. Blood samples collected from the jugular vein by syringe aspiration had a lower R and K and higher α angle than did blood samples collected from the jugular vein by evacuated tube collection. Significant differences were observed between blood samples collected from the jugular vein by syringe aspiration and samples collected from the saphenous vein by evacuated tube collection and between samples collected from the saphenous vein by evacuated tube collection and samples collected from the jugular vein through a central venous catheter.

Conclusions and Clinical Relevance—Different sampling methods resulted in small but significant differences in thromboelastographic values. Results justify the use of standardized techniques for research purposes, but all of these sampling methods were acceptable for 1-time clinical use. (Am J Vet Res 2012;73:1864–1870)
Veterinary studies have examined the preanalytic technique on routine coagulation testing. Jugular venous catheter sampling has been compared with direct jugular venipuncture in clinically normal dogs, and dogs admitted to an intensive care unit, and clinically normal cats. In canine studies, both sampling techniques produced similar results for PT, aPTT, and concentrations of fibrinogen and fibrin degradation products. In cats, there were no significant differences for PT, aPTT, or concentrations of fibrin degradation products, although fibrinogen concentration was significantly lower in jugular catheter samples. Overall, these results suggest that both sampling techniques can be used interchangeably without affecting results of plasma-based coagulation tests in cats and dogs.

The effect of preanalytic factors on thromboelastographic results in dogs has not been studied extensively. Bauer et al evaluated the effect of peripheral and CVC sites and gauge on thromboelastographic and other hemostatic variables, concluding that none had a significant effect on these tests. In another report, investigators commented on pilot data that justifies the use of jugular venipuncture and evacuated tube collection versus syringe aspiration from CVCs for their study method. However, to our knowledge, there have been no other formal studies on this subject in dogs. The goal of the study reported here was to determine whether there is a significant difference in thromboelastographic results for blood obtained by various clinically relevant collection sites and methods. Secondary aims included evaluating the effects of time between central catheter placement and blood collection and the effect of difficult phlebotomy on thromboelastography results. We hypothesized that variation in collection site and method would have no significant effect on thromboelastographic results.

**Materials and Methods**

**Dogs**—Eight adult sexually intact male dogs, including 5 Beagles ranging in body weight from 11.7 to 13.1 kg, 1 mixed-breed dog weighing 15.2 kg, and 2 Foxhounds weighing 28.0 and 29.0 kg, were used. All dogs were healthy as determined by results of physical examination, CBC, serum biochemical analysis, and urinalysis obtained 48 hours prior to commencement of the study. The dogs were concurrently enrolled in another study and undergoing acclimatization to catheter placement, blood collection, and the environment at the time of sample acquisition. The study protocol was approved by the North Carolina State University Institutional Animal Care and Use Committee.

**Preparation of dogs**—Following examination, each dog was sedated via IV administration of butorphanol (0.2 to 0.4 mg/kg) alone or in combination with midazolam (0.25 to 0.5 mg/kg) and a CVC was aseptically inserted via the Seldinger technique in the right or left external jugular vein. The catheters were inspected daily for signs of inflammation or thrombosis and flushed every 6 hours with nonheparinized saline (0.9% NaCl) solution to maintain patency. All CVC blood sampling was performed according to a described standard 3-syringe technique, whereby 5 mL of blood was aspirated from the CVC and set aside, the blood sample was collected with a separate syringe, the initial 5 mL of blood was returned, and the CVC was flushed with 2 mL of saline solution. A sample was obtained for Hct determination at least once every 48 hours to monitor for anemia.

**Sample collection**—A 20-gauge hypodermic needle with a 6-mL syringe was used to aspirate blood directly from the lateral saphenous vein, and the sample was placed into a 1.8-mL glass tube containing 3.2% sodium citrate (ratio of blood to anticoagulant, 9:1) for thromboelastographic analysis. A second sample was drawn from the contralateral saphenous vein with a 21-gauge butterfly needle via the evacuated tube collection technique. One milliliter of blood was discarded into an additive-free evacuated tube prior to filling the sodium citrate–containing tube. Following saphenous vein sample collection in all dogs and within 14 hours after collection of the first sample, a third blood sample was collected from the jugular CVC with a 20-gauge needle and 6-mL syringe and placed into a sodium citrate–containing tube. The fourth blood sample was obtained from the opposite jugular vein with a 21-gauge butterfly needle via evacuated tube collection as for the sample collected from the saphenous vein. Following sample collection for thromboelastographic analysis, 3.8 mL of blood was collected from the CVC and then placed into a 2-mL tube containing potassium EDTA and a 1.8-mL glass tube containing 3.2% sodium citrate (ratio of blood to anticoagulant, 9:1), and a coagulation panel was run. This initial sampling period for all 8 dogs was concluded within 24 hours.

The dogs were then maintained for 72 hours on an infusion of balanced electrolyte solution through the distal port of the CVC to maintain patency and mimic catheter use in a clinical setting. All CVC ports were flushed with saline solution every 6 hours. Forty-one blood samples totaling 24 mL were obtained from the CVC at intermittent time points for the purpose of evaluating baseline blood glucose and cytokine concentration measurements for the concurrent study, and a second coagulation panel was run on each dog at the end of the first day.

At 72 hours, IV fluid administration was discontinued and a replicate blood sample was obtained for thromboelastographic and coagulation panel analysis from the CVC with a 20-gauge needle and syringe aspiration. A final blood sample was collected from the contralateral jugular vein by venipuncture with a 20-gauge needle and 6-mL syringe and placed into a sodium citrate–containing tube for thromboelastographic analysis.

**Thromboelastographic analysis**—After collection, each citrate-containing tube was incubated in the upright position at 23°C for 30 minutes. Thromboelastographic analysis was performed on a system consisting of 2 integrated thromboelastographic analyzers. One milliliter of citrated blood was placed in a vial containing a kaolin activator, then inverted 5 times. A prewarmed (37°C) thromboelastographic cup was filled with 20 µL of 0.2M calcium chloride, and 340 µL of kaolin-activated, citrated blood was added within 1
minute. Paired samples, 1 aliquot on each thromboelastographic analyzer, were run concurrently from each blood sample according to manufacturer instructions. The following thromboelastographic variables were recorded for statistical comparison: R (reaction time), K (clot formation time), α angle, MA, and G (global clot strength).

Venipuncture difficulty scoring—Difficulty of phlebotomy was recorded by a single individual (JMW). A score of 0 was assigned if venipuncture was successfully performed with 1 attempt, no readjustments of the needle within the vessel were made, and there was constant blood flow into the evacuated tube or syringe. A score of 1 was assigned if venipuncture was successfully performed with 1 attempt, with slight readjustment and nearly constant flow of blood. A score of 2 was assigned if venipuncture was performed with 1 or 2 attempts, multiple readjustments in positioning of the needle were required, or there was moderate interruption in blood flow into the syringe or evacuated tube. A score of 3 was assigned if numerous attempts at venipuncture or substantial repositioning of the needle within the vessel were performed or if there was marked interruption of blood flow into the syringe or evacuated tube. All sample collections were performed by experienced veterinarians (JMW and RMH).

Coagulation profile analysis—Standard coagulation panel analysis was performed at the Clinical Pathology Laboratory of North Carolina State University and included determination of PT, aPTT, D-dimer concentration, and platelet count. Samples were delivered to the Clinical Pathology Laboratory within 15 minutes of venipuncture or substantial repositioning of the needle within the vessel. A score of 1 was assigned if venipuncture was successfully performed with 1 attempt, with slight readjustment and nearly constant flow of blood. A score of 2 was assigned if venipuncture was performed with 1 or 2 attempts, multiple readjustments in positioning of the needle were required, or there was moderate interruption in blood flow into the syringe or evacuated tube. A score of 3 was assigned if numerous attempts at venipuncture or substantial repositioning of the needle within the vessel were performed or if there was marked interruption of blood flow into the syringe or evacuated tube. All sample collections were performed by experienced veterinarians (JMW and RMH).

Order effect was examined prior to testing for significant differences in outcome variables due to sampling technique. A matched-pairs t test was used for comparison of replicate blood samples collected from the jugular vein through a CVC, and the results indicated no significant (P > 0.05) difference between the 2.

Every thromboelastographic measurement was performed in duplicate on 2 analyzers, and the mean of these technical replicates was calculated prior to analysis. Because there were no significant differences in results of the matched-pairs t tests on the replicate blood samples collected from the jugular vein through a CVC, the mean of all 4 of those catheter samples was calculated.

To test for differences in sampling methods in each of the thromboelastographic outcome variables, the Skillings-Mack test was performed. The Skillings-Mack test is a nonparametric alternative to repeated-measures ANOVA and is robust to missing data (equivalent to the Friedman test when no data are missing). A Bonferroni correction for multiple (n = 5) comparisons was applied to maintain a familywise error rate of 0.05. Values of P were corrected for multiple comparisons.

In addition to the omnibus test performed with the Skillings-Mack analyses, post hoc analysis evaluating all pairwise comparisons was performed to make inferences about which sampling methods were responsible for the significant differences. All contrasts were compared via the matched-pairs t test for each thromboelastographic outcome. To correct for multiple comparisons, a Bonferroni correction for the number of pairwise contrasts (10 pairs/thromboelastographic outcome) was applied, and values of P were corrected.

Difficulty score was dichotomized prior to analysis into difficult or not difficult categories because there were few observations with difficulty scores in the 2 or 3 categories, and then the categories were compared via Kruskal-Wallis analysis. Coagulation panel results, including PT, aPTT, D-dimer concentration, fibrinogen concentration, and platelet count, were compared for baseline and 72-hour samples via paired Wilcoxon signed rank tests. Post hoc analysis was performed with pairwise t tests to determine which contrasts were significantly different. A Bonferroni correction for the pairwise comparisons was used to control the false-positive rate.

Results

Dogs—Daily physical examination of dogs and baseline blood and urine testing revealed no clinically important abnormalities. No dog had an Hct < 35% at any time during the sampling period. Jugular venous catheters remained patent without evidence of thrombosis or phlebitis throughout the sampling period.

Effect of sampling order and analyzer channel—The order of the collection methods had no significant effect on R, K, α angle, MA, or G. Each blood sample was run in duplicate on different channels of 2 thromboelastographic analyzers. The 2 channels used for each sample produced slightly different but inconsistent (not systematic) differences in all thromboelastographic variables, and the mean of results from the 2 channels was calculated for all analyses.
Figure 1—Comparison of thromboelastographic results from various sampling methods in 8 dogs displayed according to the thromboelastographic variable: R (reaction time; A), K (clot formation time; B), α angle (C), MA (D), and G (global clot strength; E). Each box represents the interquartile range (25th to 75th percentile), and the horizontal line within each box represents the median value for each method. Whiskers represent the upper and lower limits of the range. Black circles represent outlier values (less than the first quartile minus 1.5 times the interquartile range or greater than the third quartile plus 1.5 times the interquartile range). Horizontal dashed lines represent established reference ranges as defined by Bauer et al.23 *†Values for sampling methods that are significantly (P < 0.05) different, as determined by pairwise comparison, are indicated by the same symbol. JC = Blood samples collected from the jugular vein through a CVC. JS = Blood samples collected from the jugular vein via syringe aspiration. JV = Blood samples collected from the jugular vein via evacuated tube collection. SS = Blood samples collected from the lateral saphenous vein via syringe aspiration. SV = Blood samples collected from the lateral saphenous vein via evacuated tube collection.
Jugular catheter use over time—There was no significant difference among blood samples collected from the jugular vein through a CVC for R, K, α angle, MA, or G; therefore, data from blood samples collected from the jugular vein through a CVC were combined for the remaining statistical analyses.

Comparison of all sampling techniques—The Skillings-Mack test identified the presence of significant effects of site and method for R, K, α angle, MA, and G. Post hoc Bonferroni-corrected pairwise comparisons were performed for analysis of the effects of sampling site and method on thromboelastographic variables (Figure 1).

Effect of sampling site—Sampling site (jugular vs saphenous vein) had no significant effect on R, K, α angle, MA, or G in blood samples collected by evacuated tube collection or syringe aspiration.

Effect of sampling method—There was no significant difference between blood samples collected from the saphenous vein by syringe aspiration and blood samples collected from the saphenous vein by evacuated tube collection for R, K, α angle, MA, or G. Blood samples collected from the jugular vein by syringe aspiration had significantly lower R (P = 0.015) and K (P = 0.020) and higher α angle (P = 0.004), compared with those collected by evacuated tube collection. There were no significant differences between blood samples collected from the jugular vein by evacuated tube collection and blood samples collected from the jugular vein through a CVC or between blood samples collected from the jugular vein by syringe aspiration and blood samples collected from the jugular vein through a CVC for R, K, α angle, MA, or G.

Other pairwise comparisons—Significant differences existed between other pairings of collection techniques. Blood samples collected from the jugular vein by syringe aspiration had a significantly lower K (P = 0.029) and higher α angle (P = 0.014), MA (P = 0.004), and G (P = 0.004), compared with blood samples collected from the saphenous vein via evacuated tube collection. In addition, blood samples collected from the jugular vein via a CVC had higher MA (P = 0.013) and G (P = 0.014), compared with blood samples collected from the saphenous vein by evacuated tube collection.

Effect of venipuncture difficulty on thromboelastographic variables—A significant (P < 0.001) difference was observed when difficulty scores were compared for all blood sample collection techniques, and post hoc pairwise comparisons revealed that collection from the jugular vein by syringe aspiration had a significantly higher difficulty score than did collection from the jugular vein through a CVC and collection from the jugular vein by evacuated tube collection (P = 0.018 and 0.048, respectively). Of the 48 blood samples that were collected, 30 had a difficulty score of 0, 11 had a score of 1, 5 had a score of 2, and 2 had a score of 3. Due to the small number of observations of scores > 1, difficulty scores were dichotomized into 2 groups: 1 group that had thromboelastographic results for samples with a difficulty score of 0 and 1 group that had results with difficulty scores ≥ 1. Samples with difficulty scores ≥ 1 were not significantly different from samples with difficulty scores of 0 for R, K, α angle, MA, or G.

Coagulation panels—No significant differences between the baseline and 72-hour results for PT, aPTT, D-dimer concentration, fibrinogen concentration, and platelet count were detected.

Discussion

Significant differences in thromboelastographic results were observed for blood samples collected from clinically normal dogs via different collection sites and methods. However, differences observed among techniques were small and all median values for R, K, α angle, MA, and G remained within established reference ranges for kaolin-activated thromboelastography in dogs23 as well as those established at our institution. For this reason, these results may not affect blood sampling recommendations for 1-time clinical use of thromboelastography but do suggest that standardization of blood collection methods should be considered in a research setting and for repeated analyses in a single subject.

Within each collection technique, no specific ordering strategy was used, although blood collection techniques were performed in the same order for each dog. Although sampling order would ideally have been randomized, a significant effect of order of sample collection on thromboelastographic results was not detected.

Two thromboelastographic analyzers21,f connected to a single computer interface with specialized software provided by the manufacturer were used. Four sample wells, or channels, were used between the 2 analyzers and duplicate thromboelastographic analyses were run so that 1 aliquot of blood was run on each thromboelastographic analyzer, always pairing the same channels. Analyzer channel was associated with significant but not systematic differences in thromboelastographic results, and this was handled by calculating the mean of the pairs of results.

To allow for vascular healing between repeated samplings, blood collection from the jugular vein (syringe aspiration and evacuated tube collection) was performed at 2 time points, separated by approximately 72 hours. Blood samples were obtained from the jugular venous catheter for thromboelastography and coagulation panel analysis at both time points. No significant differences were observed between the 2 collection times for coagulation panel values or any thromboelastographic variable, which suggested that the dog’s overall coagulation status as determined by thromboelastography did not change over this time period.

When all sampling techniques were compared as a whole, significant differences were observed for all thromboelastographic variables. Post hoc pairwise comparisons were used to determine which techniques had the largest contribution to these differences. When results from evacuated tube collection or syringe methods were compared between the jugular and lateral saphenous veins, no significant differences attributable to sampling site were observed.
Blood samples collected from the jugular vein by syringe aspiration had lower R and K and higher α angle and therefore were hypercoagulable, compared with blood samples collected from the jugular vein by evacuated tube collection. With vascular trauma, such as venipuncture, blood is exposed to tissue factor from perivascular tissues. The evacuated tube collection technique includes discarding of the initial portion of blood into a tube prior to acquiring the sample in a sodium citrate–containing tube. Use of venipuncture with syringe aspiration prevents the ability to discard the first portion of the sample. With the presumed presence of tissue factor in the syringe, initiation of coagulation may occur prematurely and cause results suggesting hypercoagulation. Also, fluctuations in pressure encountered with syringe manipulation during collection of blood samples from the jugular vein by venipuncture may result in excessive blood turbulence in the collection device, compared with evacuated tube collection, which provides a consistent vacuum to assist the flow of blood into the collection container. This may also contribute to premature activation of the contact pathway of coagulation. Because collection of blood samples from the jugular vein by syringe aspiration had a significantly higher difficulty score than evacuated tube collection, repeated attempts at venipuncture or repositioning of the needle may have caused an increased release of tissue factor from perivascular tissue. Consistent with this explanation, recent evidence suggests that suboptimal venipuncture may affect the results of thromboelastography in clinically normal dogs.

No other differences were found when comparing 2 methods at a single site or 2 sites for a single collection method; however, disparities in venipuncture difficulty or alterations in blood flow between saphenous and jugular veins may have minimized detectable differences in thromboelastographic results. For example, hypercoagulability induced by venipuncture in blood samples collected from the jugular vein by evacuated tube collection may have been offset by a more laminar flow during blood sample collection, compared with blood samples collected from the jugular vein through a CVC. The influence of vessel anatomy, difficulty of sample acquisition, and flow of the blood sample during collection may interact in a complex manner.

Blood samples collected from the jugular vein by syringe aspiration were hypercoagulable (lower K and higher α angle, MA, and G) in comparison with blood samples collected from the saphenous vein by evacuated tube collection. Jugular catheter samples also had higher MA and G, suggesting that they were hypercoagulable relative to blood samples collected from the saphenous vein by evacuated tube collection. A combination of factors, including vessel size and sample turbulence, may have led to the differences observed between these methods.

Sedation with butorphanol with or without midazolam was performed to facilitate CVC placement. The authors are not aware of any studies documenting coagulation abnormalities following butorphanol administration. Within the past decade, research indicates that midazolam administration may reduce platelet aggregation, considering the antithrombotic properties in human patients. The relevance of these findings to canine patients is currently unknown.

Needles of 2 gauges, including a 20-gauge hypodermic needle for syringe techniques and a 21-gauge butterfly needle for evacuated tube collection techniques, were used for venipuncture. These needle sizes were chosen on the basis of similar size, product availability, and use in our hospital. Use of the smaller (21-gauge) needle may have contributed to a reduced rate of blood flow into the collection device and a different level of turbulence in the blood sample, compared with the 20-gauge needle. Ideally, an identical gauge would have been used for needles throughout all sampling techniques to minimize variation in blood flow due to needle size.

Saphenous vein blood sampling was performed immediately after sedation and CVC placement, whereas dogs were fully recovered from the sedation event for other sampling methods. Sedation may have reduced venipuncture difficulty; indeed, the highest level of difficulty (3) was observed in only 2 samples. This study was not designed to evaluate the effect of traumatic venipuncture on thromboelastographic results, and any conclusions regarding this are further limited by the small number of samples (n = 7) with difficulty scores of 2 and 3.

Between the 2 sampling days, dogs underwent IV fluid administration and intermittent blood sampling through the jugular catheters. Blood sampling, removing up to 24 mL (≤ 2 mL/kg), was performed for the purpose of establishing baseline values in a different study, and blood loss (≤ 2.2% of blood volume) from this procedure was considered minimal. Fluids were administered IV to simulate normal clinical use of indwelling jugular catheters and to maintain catheter patency. The authors are not aware of any study documenting hemostatic consequences of administering an isotonic crystalloid fluid at a rate of 80 to 100 mL/kg/d in clinically normal dogs without evidence of dehydration or hypovolemia. Dilutional coagulopathy is known to occur in patients with hemorrhagic shock and rapid intravascular volume replacement; however, this condition has not been described with slow crystalloid fluid administration in euvolemic animals.

Both anemia, which causes reduced blood viscosity, and thrombocytopenia have been reported to affect thromboelastographic results. The authors do not believe that these conditions were a factor in the study reported here, considering that no dog’s Hct decreased to < 35% and clinically important thrombocytopenia was not encountered. It is also noteworthy that the coagulation profiles did not substantially change over time. A limitation of this study was that a low number of dogs were included. Substantial effects on thromboelastographic results may have been more or less pronounced with a larger number of dogs. This study was performed on healthy dogs to avoid the confounding factors of various medical conditions that may occur in a clinical setting. The effects of sampling methodology may be attenuated or magnified in a diseased or critically ill population.

In the present study, the results of thromboelastography in clinically normal dogs differed significantly when various sampling methods were compared. How-
ever, differences observed remained within established reference ranges. The authors believe this finding should prompt investigators to standardize their phlebotomy technique in a research setting, and blood collection techniques in a clinical setting should be consistently used for patients undergoing repeated blood sampling in an effort to monitor changes in thromboelastographic results. When considering the use of thromboelastography to assess coagulation in a single patient at 1 point in time, it appears to be acceptable to use any of the different phlebotomy techniques for collection of blood samples. If different collection sites must be compared, blood sampling via needle and syringe or evacuated tube collection appeared to provide consistent results for different sampling sites. Withdrawal of blood from a sampling catheter, at least when done within 3 days after catheter placement, appears to be an acceptable sampling method and facilitates collection of blood without major stress, signs of pain, or need for restraint. Further study is needed to investigate the effects of sampling method on thromboelastographic results in hospitalized or critically ill patients.

References