Effect of citrate concentration on coagulation test results in dogs

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Objective—To determine the effect of citrate concentration (3.2 vs 3.8%) on coagulation tests in dogs.

Design—Original study.

Animals—30 clinically healthy dogs and 12 dogs with hereditary hemostatic disorders.

Procedure—Blood was collected from all dogs directly into collection tubes containing 3.2 or 3.8% buffered citrate. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentration were measured by use of 3 clot-detection assay systems (2 mechanical and 1 photo-optic). Factor VIII and factor IX coagulant activities (FVIII:C and FIX:C, respectively) were determined by use of a manual tilt-tube method and a mechanical clot-detection device.

Results—Significant differences were not detected in median PT, fibrinogen concentration, FVIII:C, or FIX:C between 3.2 and 3.8% citrate for any assay system. A significant prolongation in aPTT for 3.2% citrate, compared with 3.8% citrate, was found in 1 mechanical system.

Conclusions and Clinical Relevance—Citrate concentration does not significantly affect results of most coagulation assays, regardless of assay system. The aPTT was mildly influenced by the citrate concentration, although this was animal-, instrument-, and reagent-dependent. The choice of 3.2 or 3.8% citrate as an anticoagulant for coagulation tests has minimal influence on assay results in healthy dogs or dogs with hereditary hemostatic disorders. (*J Am Vet Med Assoc* 2000;217:1672–1677)

Coagulation testing is routinely performed in dogs with clinical signs of hemorrhage or diseases known to impair hemostasis (eg, liver failure, hemangiosarcoma). Coagulation assays are also used as screening tests for detection of hereditary hemostatic disorders. The most commonly used coagulation screening tests are **prothrombin time (PT)**, **activated partial thromboplastin time (aPTT)**, and thrombin clotting time or fibrinogen concentration. Coagulation-factor deficiency or inhibition is detected through prolongations of clotting time in 1 or more of these assays.[1,2]

There are numerous variables other than disorders of hemostasis that will affect the results of coagulation screening tests. These variables are classified as preanalytic, analytic, and postanalytic. Preanalytic variables such as sample collection, handling, and storage have the greatest impact on coagulation results. Coagulation factors can be activated or consumed during traumatic venipuncture, and the correct citrate-to-blood ratio must be maintained to prevent inadequate or excessive calcium chelation with consequent artifactual changes of clotting times in vitro.[1-4] Analytic variables include the reagents and clot-detection instruments used to perform coagulation assays. There are several instruments available; the most widely used are based on mechanical or photo-optic clot-detection methods. The choice of reagent and clot-detection instrument influences the sensitivity of these screening assays to coagulation-factor deficiencies.[1,2,5,6] Postanalytic variables such as data entry and reporting typically have the least effect on results.

Collection of blood samples into sodium citrate anticoagulant is required for coagulation testing. The concentration of citrate, however, is not usually considered a preanalytic variable. The standard citrate concentration used in the United States is 3.8% (0.129 mol/L), whereas 3.2% (0.109 mol/L) is the standard concentration used in Europe, Australia, and the United Kingdom.[7] Recently, several studies have revealed that citrate concentration affects the results of coagulation assays in human patients, especially those receiving anticoagulants for prevention of thrombosis. Some authors recommend the use of a uniform citrate concentration for coagulation assays and consider 3.2% optimal, because it is more isotonic with plasma.[8-12] The World Health Organization recently recommended the use of 3.2% citrate for monitoring anticoagulant treatment in humans.[11]

Veterinarians in the United States routinely use 3.8% citrate as the anticoagulant for coagulation testing in dogs. With the possibility that only sample collection tubes containing 3.2% citrate will be available for coagulation testing (if manufacturers discontinue production of 3.8% citrate tubes), there is a need to determine whether citrate concentration affects coagulation assays in dogs. One study in 12 healthy dogs did not reveal significant changes in PT, aPTT, or thrombin clotting time in samples containing the equivalent of 3.2 or 3.8% citrate.[3] However, the 3.2% citrate concentration in that study was obtained by altering the citrate-to-blood ratio of 3.8% citrate (from 1:9 for 3.8% to 1:11 for 3.2%), and dogs with hereditary hemostatic disorders were not evaluated.

The purpose of the study reported here was to determine whether variation in citrate concentration in sample collection tubes significantly alters the results of routine coagulation tests (PT, aPTT, and fibrinogen concentration) and factor VIII and IX coagulant activi-

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ties (FVIII:C, FIX:C, respectively) in healthy dogs and dogs with hereditary hemostatic disorders. Furthermore, we wanted to determine whether any detectable citrate effect was assay-system (reagent-instrument) dependent.

Materials and Methods

Thirty clinically healthy dogs and 12 dogs with hereditary hemostatic disorders were evaluated in this study. Factor deficiencies included hemophilia A (n = 4), hemophilia B (4), and **von Willebrand disease (vWD**; type 1 vWD, 1; type 2 vWD, 1; type 3 vWD, 2). Dogs with hemophilia, dogs with type 2 and 3 vWD, and 2 healthy dogs were obtained from a research colony at Cornell University. These dogs were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility, and blood collection procedures were followed according to an approved Institutional Animal Care and Use Committee protocol. The remaining dogs (29) were veterinary student- and faculty-owned, and owners' informed consent was obtained before blood collection.

Blood was collected from each dog through a butterfly catheter from a single venipuncture site into 2 collection tubes: 1 containing 3.2% citrate and 1 containing 3.8% citrate.[a] A coin toss determined the concentration in the first tube for any dog. Sample volume per tube was 5.0 ml, and a 1:9 citrate-to-blood ratio was maintained. Platelet-poor plasma was separated after centrifugation at 1,000 × g for 10 minutes. Plasma was stored at 4 C and analyzed within 4 hours of collection or frozen at or below –25 C before being analyzed in batches within 1 month of collection. Frozen samples were thawed in a 37 C water bath before analysis.

Coagulation tests (PT, aPTT, and fibrinogen concentration) were performed on paired plasma samples collected into 3.2 and 3.8% citrate tubes from all dogs. Three clot-detection instruments were used: Fibrometer[b] and STA Compact[c] (mechanical endpoint detection) and Coag-A-Mate XM[d] (photo-optic endpoint detection). Factor activities (VIII:C and FIX:C) were measured in 11 healthy dogs and all dogs with hemophilia A and B. Factor IX:C was not measured in dogs with vWD. Two assay systems were used to measure factor activities: a manual method[13] and a mechanical instrument (ST4[c]).

In the Fibrometer assay system, rabbit brain phospholipid[f] reconstituted with 0.025 mol/L calcium was used to measure PT. The aPTT was measured, using a rabbit brain phospholipid and micronized silica reagent[g] and an activation time of 5 minutes. Fibrinogen concentration was determined in a 1:10 dilution of plasma, using the Clauss method[14] with a 100-NIH U/ml bovine thrombin reagent.[h] A standard curve was derived from dilutions (1:5, 1:10, 1:15, 1:25, and 1:40) of a human plasma standard[i] with the manufacturer's assigned value of 316 mg of human fibrinogen/dl . Intra-assay coefficients of variation (determined from 10 measurements of pooled canine plasma in 3.8% citrate) for the PT, aPTT, and fibrinogen concentration were 4.6, 2.1, and 1.9%, respectively.

For the STA Compact system, PT was measured, using a rabbit brain phospholipid reagent[j] diluted 1:4 with buffer and an incubation time (before the addition of 0.025 mol/L calcium to the reaction mixture) of 30 seconds. The aPTT was measured, using a soy phosphatide phospholipid and an ellagic acid activator[k] and an activation time of 3 minutes. Fibrinogen concentration was measured in a 1:20 dilution of plasma, using the Clauss method and a 100-NIH U/ml human thrombin reagent.[l] A standard curve was derived from dilutions (1:6, 1:10, 1:20, 1:30, and 1:60) of pooled normal canine plasma containing 386 mg of canine fibrinogen/dl, the concentration of which was determined by the gravimetric method.[15] Intra-assay coefficients of variation for the PT, aPTT, and fibrinogen concentration were 2.3, 3.6, and 2.2%, respectively.

In the Coag-A-Mate XM assay system, the PT reagent[f] was the same as that used for the Fibrometer. The aPTT was measured, using a purified bovine phospholipid and silica-type activator reagent[m] and an activation time of 5 minutes. Quantitative determination of fibrinogen was performed on a 1:10 dilution of plasma, using the Clauss method and a 100-NIH U/ml bovine thrombin reagent.[n] A standard curve was derived from serial dilutions (1:5 to 1:40) of a human plasma standard[o] with the manufacturer's assigned value of 356 mg of human fibrinogen/dl. The manufacturer's recommended reagents, sample and reagent volumes, and default settings for incubation and activation times were used for all tests in this system. The instrument blank times (lag time from addition of reagent until monitoring of clot formation) were modified from the default setting for PT (from 7 to 4 seconds) and aPTT (from 20 to 1 second). These modifications were required because clotting times are faster in dogs than humans.[2] Intra-assay coefficients of variation for the PT, aPTT, and fibrinogen concentration were 1.6, 1.6, and 3.5%, respectively.

Factor VIII:C and FIX:C were measured by use of a modified 1-stage aPTT assay[13] with a manual tilt-tube method or mechanical clot-detection device (ST4). For both assay systems, the aPTT was performed, using a rabbit brain cephalin and silica-type activator reagent[p] combined with a dilute kaolin[q] solution (10 mg/ml) and an activation time of 10 minutes. The substrate was FVIII- or FIX-deficient plasma obtained from dogs with severe hemophilia A or B, respectively. After log-log transformation, the clotting times of test plasma samples were reported as a percentage of FVIII:C or FIX:C, compared with dilutions of pooled normal canine plasma having an assigned value of 100% FVIII:C and FIX:C, respectively. Intra-assay coefficients of variation for FVIII:C and FIX:C were 3.0 and 5.0% (manual system) and 7.8 and 4.0% (ST4 assay system).

Statistical analyses—For each assay system, median values of each test (PT, aPTT, fibrinogen concentration, FVIII:C, and FIX:C) for samples collected into 3.2 and 3.8% citrate were compared, using the 2-tailed Wilcoxon signed-rank test for paired data[r] for each group of dogs separately (healthy, hemophilia A, hemophilia B, and vWD). For each assay system, Spearman rank correlations[r] for the association between 3.2 and 3.8% citrate were determined for each test (only in healthy dogs; there were insufficient numbers of factor-deficient dogs to obtain correlations.)

To compare the magnitude of difference between paired samples in various assay systems, the median difference between results for 3.2 and 3.8% citrate for each assay system was calculated as follows: result in 3.2% citrate minus result in 3.8% citrate. Results for factor-deficient dogs were combined for this purpose because of the low number of dogs with each defect. Median difference for the PT, aPTT, and fibrinogen concentration was compared, using the Friedman 2-way ANOVA, followed (as needed) by the 2-tailed Wilcoxon signed-rank test. The median difference for FVIII:C and FIX:C was compared, using the 2-tailed Wilcoxon signed-rank test. Values of $P < 0.05$ were considered significant.

Results

The PT results in 12 samples from 8 healthy dogs and 1 dog with vWD (paired plasma samples, n = 3; 3.2% citrate sample only, 4; 3.8% citrate sample only, 2) could not be measured with the Coag-A-Mate XM system, because the instrument failed to detect a clot endpoint. These dogs were excluded from analyses of

the Coag-A-Mate XM system. Significant differences were not detected in any assay system between the median PT of healthy or factor-deficient dogs for samples collected into 3.2 or 3.8% citrate (**Table 1**). The Spearman rank correlations for healthy dogs were 0.89 for the Fibrometer system and 0.87 for the STA Compact and Coag-A-Mate XM systems ($P < 0.05$ for all correlations). There was a 0- to 1-second difference between paired samples in individual dogs (healthy and factor-deficient) with all assay systems. The median difference in paired samples was not significantly different between the 3 assay systems for either the healthy or factor-deficient dogs.

Significant differences were not detected between the median aPTT of healthy or factor-deficient dogs for samples collected into 3.2 or 3.8% citrate, using the STA Compact and Coag-A-Mate XM assay systems (**Table 2**). The median aPTT for healthy dogs was significantly longer in samples collected into 3.2% citrate with the Fibrometer assay system. Correlations for healthy dogs were 0.85 for the Fibrometer system, 0.89 for the STA Compact system, and 0.90 for the Coag-A-Mate XM system ($P < 0.05$ for all correlations). Comparisons between aPTT for paired samples revealed slight (1 to 2 second) differences for healthy dogs and dogs with vWD with all assay systems. The hemophilic dogs, however, had more variable results in all systems, with a maximum observed difference of 25 seconds for 1 dog with hemophilia B in the Coag-A-Mate XM system. The median difference in paired samples from healthy dogs was significantly different with the Fibrometer, compared with the other assay systems.

Significant differences were not detected between median fibrinogen concentration of healthy or factor-deficient dogs for samples collected into 3.2 or 3.8% citrate, using any assay system (**Table 3**). Correlations for healthy dogs were 0.97 for the Fibrometer system, 0.86 for the STA Compact system, and 0.72 for the Coag-A-Mate XM system ($P < 0.05$ for all correlations). There were large differences between fibrinogen concentrations in paired samples (ranging from 11 to 147

Table 1—Prothrombin times (seconds) in 3.2 and 3.8% citrated plasma samples from 30 healthy dogs and 12 dogs with hereditary hemostatic disorders (4 dogs in each group), using 2 mechanical (Fibrometer and STA) and 1 photo-optic (Coag-A-Mate XM) clot-detection devices

	Fibrometer		STA		Coag-A-Mate XM*	
Dog group	**3.2**	**3.8**	**3.2**	**3.8**	**3.2**	**3.8**
Healthy						
Median	7	7	17	17	8	8
Range	6–8	6–8	14–19	14–19	7–9	7–9
Hemophilia A						
Median	7	7	15	15	8	7
Range	7–7	6–7	15–17	14–17	7–8	7–8
Hemophilia B						
Median	7	7	19	19	8	8
Range	6-8	6–8	16–20	16–20	8–9	8–9
vWD						
Median	7	7	17	18	8	8
Range	6–7	6–8	16–20	15–20	7–8	7–8

*For healthy dogs, n = 23 and 26 for 3.2 and 3.8% citrate, respectively. For dogs with vWD, n = 3 for 3.8% citrate only. vWD = von Willebrand disease.

Table 2—Activated partial thromboplastin times (seconds) in 3.2 and 3.8% citrated plasma samples from 30 healthy dogs and 12 dogs with hereditary hemostatic disorders (4 dogs in each group), using 2 mechanical (Fibrometer and STA) and 1 photo-optic (Coag-A-Mate XM) clot-detection devices

	Fibrometer		STA		Coag-A-Mate XM	
Dog group	**3.2**	**3.8**	**3.2**	**3.8**	**3.2**	**3.8**
Healthy						
Median	15*	14	14	14	10	10
Range	12–20	12–18	12–19	12–18	7–14	8–13
Difference†	0 (–1 to 2)		–0.4 (–2 to 1)		–0.3 (–1 to 1)	
Hemophilia A						
Median	26	24	27	30	19	20
Range	21–26	23–28	21–31	21–33	17–20	17–21
Hemophilia B						
Median	41	38	43	46	43	46
Range	30–46	35–43	28–55	28–56	30–70	30–95
vWD						
Median	16	16	15	15	9	10
Range	11–19	12–17	13–16	13–17	8–13	7–13
Difference†	2 (–6 to 6)		–1 (–5 to 2)		–1 (–25 to 2)	

*Result in 3.2% citrate significantly ($P < 0.05$) different from result in 3.8% citrate. †Difference between results (expressed as median and [range]) obtained from 3.2 and 3.8% in the same dog. The results from factor-deficient dogs were combined. In the healthy dogs, the result for the Fibrometer system was significantly ($P < 0.05$) different from that of the STA Compact or Coag-A-Mate XM systems.
vWD = von Willebrand disease.

Table 3—Fibrinogen concentration (mg/dl) in 3.2 and 3.8% citrated plasma samples from 30 healthy dogs and 12 dogs with hereditary hemostatic disorders (4 dogs in each group), using 2 mechanical (Fibrometer and STA) and 1 photo-optic (Coag-A-Mate XM) clot-detection devices

	Fibrometer		STA		Coag-A-Mate XM	
Dog group	**3.2**	**3.8**	**3.2**	**3.8**	**3.2**	**3.8**
Healthy						
Median	174	176	316	334	161	158
Range	106–270	109–260	131–545	152–498	59–278	77–263
Hemophilia A						
Median	258	248	504	469	178	183
Range	230–313	230–313	453–569	353–541	172–278	145–250
Hemophilia B						
Median	187	187	413	405	153	149
Range	159–260	159–253	376–551	329–530	87–264	96–262
vWD						
Median	328	324	636	628	291	271
Range	131–481	135–502	231–851	275–845	109–368	112–351

vWD = von Willebrand disease.

mg/dl) from healthy and factor-deficient dogs with all assay systems; the greatest differences were found with the STA Compact system. However, the median difference in fibrinogen concentration between paired 3.2 and 3.8% samples was not significantly different between the assay systems.

Significant differences were not detected between median FVIII:C or FIX:C of healthy or factor-deficient dogs for samples collected into 3.2 or 3.8% citrate with either assay system. Correlations for FVIII:C were 0.82 ($P = 0.002$) and 0.74 ($P = 0.01$) for the manual method and ST4 system, respectively. Correlations for FIX:C were 0.70 ($P = 0.02$) and 0.73 ($P = 0.01$) for the manual method and ST4 system, respectively. Differences between paired samples for the specific factors ranged between 0 and 2% in dogs with hemophilia A and B. Differences in paired samples from healthy dogs and dogs with vWD were much larger (ranging between 0 and 39%); the greatest difference was detected for FVIII:C with the ST4 system. However, the median difference in paired samples for FVIII:C or FIX:C with the 2 assay systems was not significantly different for healthy or factor-deficient dogs.

To examine the effects of differing citrate concentrations on interpretation of individual test results, reference intervals for each citrate concentration were established, using the results of the healthy dogs in this study. Because data were not normally distributed, reference intervals were determined from the upper and lower limits of the results. Individual results (in each citrate concentration) of coagulation tests from factor-deficient dogs were compared with both (citrate-specific) reference intervals. Reference intervals for PT were identical for both citrate concentrations in all 3 assay systems. The PT of factor-deficient dogs were within reference intervals with the Fibrometer and Coag-A-Mate XM systems. With the STA Compact system, 1 dog with hemophilia A and 1 dog with vWD had a slight prolongation of the PT (0.5 to 1.4 seconds in both citrate concentrations). The aPTT of paired samples was prolonged in all dogs with hemophilia A and B with all assay systems. The aPTT of paired samples from dogs with vWD were within reference intervals for the STA and Coag-A-Mate XM systems. For the Fibrometer system, the aPTT of 1 dog with vWD in 3.2% citrate was within the reference interval for 3.2% citrate but was 1 second longer than the reference interval for 3.8% citrate. Fibrinogen concentration was high (compared with both reference intervals) in paired samples from 2 dogs with vWD with all 3 assay systems. Fibrinogen concentration was also high (compared with both reference intervals) in paired samples from 2 dogs with hemophilia (1 dog with hemophilia A with the Fibrometer and STA Compact systems and 1 dog with hemophilia B with the STA Compact system). As expected, FVIII:C and FIX:C were considerably decreased in both citrate concentrations in the dogs with hemophilia A and B, respectively (data not shown). Factor VIII:C was mildly decreased (compared with both reference intervals) in paired samples from 3 dogs with vWD with the manual method (FVIII:C; range, 29 to 44%) and in paired samples from 1 dog with vWD with the ST4 system.

Discussion

Analysis of our results revealed there is good correlation between the results of coagulation tests from blood samples collected in 3.2 or 3.8% citrate, regardless of assay system. The 3 instruments used in this study represented 3 methods of clot detection. The Fibrometer is a mechanical instrument that uses 2 electrodes (1 stationary and 1 mobile) to detect fibrin clot formation. As fibrin forms in the reaction mixture, it completes an electrical circuit (between the moving electrode, the sample, and the stationary electrode) that shuts off electrode movement and triggers a timing device. The STA Compact is a fully automated mechanical system that detects fibrin formation by the change in oscillation amplitude of a metal ball caused by increased sample viscosity as a fibrin clot forms (the metal ball is maintained in a constant pendular swing by an electromagnetic field applied on either side of the reaction cuvette). The Coag-A-Mate XM is a photooptic instrument that detects clot formation by monitoring the rate of change in light transmission that develops when fibrinogen is converted to fibrin. Apparently, variation in citrate concentration does not significantly affect the rate change of sample viscosity or turbidity that these coagulation instruments use to detect clot formation.

Analysis also indicated that, using the assay systems evaluated in this study, the PT, fibrinogen concentration, FVIII:C, and FIX:C of blood samples collected into 3.2 or 3.8% citrate can be interpreted, using reference intervals established from samples collected into either citrate concentration. The aPTT was significantly longer in 3.2%, compared with 3.8%, citrate in healthy dogs in the Fibrometer system, which suggests that this technique is sensitive to differences in citrate concentration. These results indicated that, for this assay system, reference intervals should be derived from samples collected into 3.2 and 3.8% citrate. Alternatively, citrate concentration-specific reference intervals could be used. In human patients, the effect of citrate concentration on coagulation tests is similarly reagent- and instrument-dependent.[8] In a previous study in healthy Beagles, aPTT was more sensitive than PT (ie, the aPTT became longer as citrate concentration increased) to altered citrate-to-blood ratio, especially if blood was collected into 3.8% rather than 3.2% citrate.[4] The actual difference between the aPTT results of samples collected into 3.2 and 3.8% citrate from healthy dogs with the Fibrometer assay system was small (ranging from 1 second shorter to 2 seconds longer in 3.2% citrate in the same dog) but was greater than can be attributed to analytic variation in 7 dogs (ie, in those with a 2-second difference). The aPTT is sensitive to activation of factor X, which can develop during sample collection,[16] and some of the differences in our study may have been attributable to collection technique. However, collection artifact was minimized in our study by using a butterfly catheter to collect blood into both citrate collection tubes from a single venipuncture site and by randomizing the order in which blood was collected into the various citrated collection tubes. Furthermore, the largest difference in the aPTT result was found when blood was collected first

and when collected last into 3.2% citrate, suggesting that this concentration does result in longer aPTT results in some dogs, using the Fibrometer assay system. Although the increase in the aPTT was small, the change could be clinically relevant, because aPTT results of some dogs with hemophilia A were prolonged (compared with results of healthy dogs in this study) by only 1 to 2.5 seconds with Fibrometer and STA Compact assay systems.

There were considerable differences in individual and group results for PT, aPTT, and fibrinogen concentration between the 3 assay systems. These differences were independent of citrate concentration, which emphasizes that results obtained with different assay systems should not be directly compared. Interpretation of individual test results must be made in the context of reference intervals established for each assay system. Comparisons between assay systems in their sensitivity to factor deficiencies were beyond the scope of this study. Obvious differences between systems, however, were evident in the PT and fibrinogen assays. The Coag-A-Mate XM system failed to detect clot formation in the PT assay in 12 of 84 samples. Visual inspection of the reaction cuvettes revealed that a fibrin clot had formed in each of these samples. In photooptic clot-detection instruments, the rate of change in light transmission detected during clot formation is converted by a predetermined algorithm (set for each instrument to determine a clotting time). Parameters for threshold and duration of light transmission are developed and optimized for detection of fibrin clot formation in humans. It is likely that the kinetics of clot formation in PT assays in plasma samples from some dogs differ from the instrument's predetermined algorithm, preventing detection of a clot. All dogs had a longer PT with the STA Compact system, because the PT reagent was diluted with buffer before addition of the sample. The higher individual and median fibrinogen concentrations measured, using the STA Compact system, compared with the Fibrometer and Coag-A-Mate XM assay systems, can be attributed to the use of a canine-based calibrator (rather than a human-based calibrator) for generation of the standard curve. The effects of differing clot-detection instruments and calibrators were also apparent in comparisons between fibrinogen determinations with the Fibrometer and Coag-A-Mate XM assay systems. Although both use human-based calibrators, the individual values, group medians, and reference intervals from the healthy dogs were obviously different.

The citrate concentration in sample collection tubes did not affect interpretation of individual results from factor-deficient dogs, despite the large differences that were detected in many paired samples (especially with measurement of aPTT and fibrinogen). As expected in this study population, the PT and fibrinogen values did not indicate extrinsic or common pathway factor deficiencies in any dogs. The aPTT, however, is sensitive to intrinsic system factor deficiencies and is expected to be prolonged in dogs with hemophilia A and B. Sensitivity of aPTT to deficiencies of these factors depends on the instrumentation and reagents.[2] In all dogs with hemophilia in this study, aPTT results were prolonged in both citrate concentrations, compared with reference intervals established from results of the healthy dogs. Dogs with hemophilia B had consistently longer aPTT results than those with hemophilia A in both citrate concentrations with all 3 assay systems. This may be attributable to their more severe factor deficiency (< 1% FIX:C) and greater sensitivity of the aPTT procedure to FIX versus FVIII deficiency.[6] The aPTT results in dogs with vWD were primarily within reference intervals, although some of these dogs had mildly decreased FVIII:C. Mild FVIII deficiency is a common feature of vWD in dogs,[17] although values rarely decrease < 30% (the value at which aPTT would be expected to be prolonged). The increased fibrinogen concentration in several factor-deficient dogs was attributed to an acute-phase reactant response to inflammation or recent hemorrhage. The STA Compact system detected increased fibrinogen concentrations in more dogs than the Fibrometer or Coag-A-Mate XM assay systems, suggesting that the canine calibrator-based STA Compact system is more sensitive to increases in fibrinogen concentration.

The effect of citrate concentration, although minimal in this study of healthy dogs and dogs with hereditary hemostatic disorders, may be more pronounced in dogs treated with anticoagulants. Citrate concentration has a minor effect on coagulation assays in healthy humans, with differences dependent on reagents and instrumentation.[8,9,11] However, differences are more pronounced in patients undergoing anticoagulant treatment orally.[8,10,11] In the latter patients, PT and aPTT are longer for samples collected into 3.8%, compared with 3.2%, citrate, findings that are not consistent with our results. The **international sensitivity index (ISI)** is a measure of the sensitivity of the PT thromboplastin reagent to anticoagulants, compared with an international reference plasma calibrated by the World Health Organization (using a manual tilt-tube method and 3.2% citrate). The PT is longer with low ISI reagents; thus, these reagents are more sensitive to anticoagulants. The ISI is used to calculate **international standardized ratios (INR)**, a data transformation designed to standardize and simplify monitoring of human patients on anticoagulant treatment.[11,18] Both the ISI and INR are affected substantially by citrate concentration.[8,10-12] Although the INR system is not used to regulate induction of anticoagulant treatment or diagnose bleeding disorders in humans, it is recommended for managing long-term anticoagulant treatment.[18] In our study population, the use of 3.2 or 3.8% citrate in sample collection tubes had minimal effect on PT results. However, citrate concentration in collection tubes may be important for monitoring anticoagulant treatment in dogs, especially if the INR is used for reporting results.[19]

[a]Vacutainer, Becton-Dickinson Co, Franklin Lakes, NJ.
[b]BBL Fibrosystem, Becton-Dickinson, Cockeysville, Md.
[c]STA Compact, American Bioproducts, Parsippany, NJ.
[d]Coag-A-Mate XM, Organon Teknika, Durham, NC.
[e]ST4, American Bioproducts, Parsippany, NJ.
[f]Simplastin Excel, Organon Teknika, Durham, NC.
[g]Automated APTT, Organon Teknika, Durham, NC.
[h]Thrombin Reagent, Dade International, Miami, Fla.
[i]Fibrinogen Calibration Reference for mechanical instruments, Dade International, Miami, Fla.

[j]Thromboplastin L, Pacific Hemostasis, Huntersville, NC.
[k]Dade Actin FS, Baxter Diagnostics, Edison, NJ.
[l]STA Fibrinogen, American Bioproducts, Parsippany, NJ.
[m]Platelin L, Organon Teknika, Durham, NC.
[n]Fibriquick, Organon Teknika, Durham, NC.
[o]Fibrinogen Calibration Reference, Organon Teknika, Durham, NC.
[p]Dade Actin, Baxter Diagnostics, Edison, NJ.
[q]Kaolin, Fisher Scientific, Pittsburgh, Pa.
[r]Analyse-it, version 1.44, Analyse-it Software Ltd, Leeds, England.

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