Evaluation of a canine D-dimer point-of-care test kit for use in samples obtained from dogs with disseminated intravascular coagulation, thromboembolic disease, and hemorrhage

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Objective—To evaluate a canine D-dimer point-of-care (cD-d POC) test kit for use in healthy dogs and dogs with disseminated intravascular coagulation (DIC), thromboembolic disease (TED), and hemorrhage.

Animals—12 healthy dogs, 18 dogs with DIC, 23 dogs with TED (19 acute and 4 chronic), and 18 dogs with hemorrhage.

Procedure—The cD-d POC, canine D-dimer ELISA (cD-d ELISA), human D-dimer latex agglutination (hD-d LA), and fibrin degradation product (FDP) tests were performed on citrated plasma.

Results—All healthy dogs had negative cD-d POC test results and mean cD-d ELISA value of 0.2 U/mL. All dogs with DIC had positive cD-d POC test results and mean cD-d ELISA value of 44 U/mL. Dogs with acute TED had a mean cD-d ELISA value of 34 U/mL, and 17 of 19 had positive cD-d POC test results. Mean cD-d ELISA value in dogs with hemorrhage was 14 units/mL, and 15 of 18 had positive cD-d POC test results. The cD-d ELISA values in dogs with hemorrhage were significantly higher than those of healthy dogs but lower than those of dogs with DIC and acute TED. The cD-d POC, cD-d ELISA, and hD-d LA tests were comparable in differentiating healthy dogs from dogs with DIC, acute TED, or hemorrhage and appeared to be superior to measurement of FDPs.

Conclusions and Clinical Relevance—The cD-d POC test kit can be quickly and easily used and reliably detects dogs with DIC or acute TED. Positive results may also be seen in dogs with internal hemorrhage. (*Am J Vet Res* 2003;64:1562–1569)

During the process of clot formation, prothrombin is converted to thrombin, which cleaves plasma fibrinogen to form soluble fibrin monomers. The fibrin monomers then polymerize and are covalently bound by factor XIII to produce insoluble cross-linked fibrin. Fibrinogen and fibrin monomers are composed of 2 large terminal D-domains and a smaller central E-domain. During conversion of fibrin monomers to cross-linked fibrin, the D-dimer is formed by cross-linking the D-domains of 2 adjacent fibrin monomers (**Fig 1**). During fibrinolysis, plasmin cleaves fibrinogen and cross-linked fibrin. Cleavage of fibrinogen results in a series of products including fragments X, Y, D, and E.[1] Cleavage of cross-linked fibrin releases these same fragments as well as newly formed cross-linked fragments (or X-oligomers),[2] the smallest of which is the DDE fragment.[3]

Clinical assessment of fibrinolysis has traditionally included measurement of **fibrin degradation products (FDPs)**, which are also known as fibrin split products. Most commercial FDP assays measure fragments D and E in plasma[4]; thus, plasma concentrations of FDP are theoretically increased by degradation of fibrin and fibrinogen. However, fibrinogen degradation in vivo is rare in human patients and has not been documented in dogs. More recently, D-dimer assays, which recognize the D-dimer within X-oligomers and therefore are specific for degradation of cross-linked fibrin,[5] have been developed. Increased plasma concentrations of FDPs and D-dimer are expected in hypercoagulable conditions such as **disseminated intravascular coagulation (DIC)**[5,6] and other **thromboembolic diseases (TEDs)**,[7,a] but they may also be detected in association with degradation of any other blood clot. Blood D-dimer assays are currently considered sensitive diagnostic tests for monitoring DIC[5,8] and TED[9] in humans; however, they are not specific for DIC and TED.

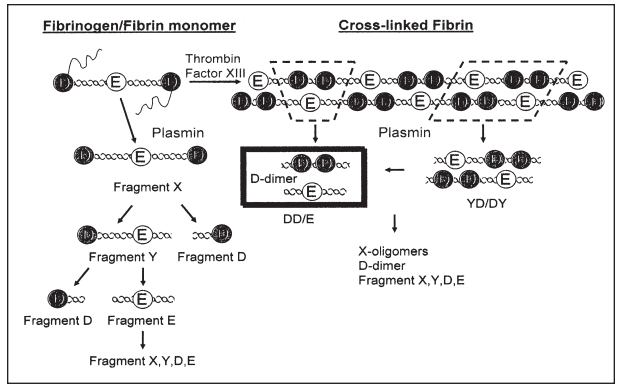

Figure 1—Schematic depicting formation of D-dimer by cross-linking of the terminal D-domains of 2 adjacent fibrin monomers. Cleavage of fibrinogen results in a series of products including fragments X, Y, D, and E, and cleavage of cross-linked fibrin releases the same fragments in addition to the newly formed cross-linked fragments (X-oligomers) containing D-dimer. (Modified from Marder VJ, Francis CW, Doolittle RF. Fibrinogen structure and physiology. In: Colman RW, Hirsh J, Marder VJ, et al, eds. *Hemostasis and thrombosis: basic principles and clinical practice*. Philadelphia: Lippincott Williams & Wilkins, 1982;145–163. Reproduced with permission.)

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Although negative results for blood D-dimer tests make DIC[5] and TED[10] highly unlikely in humans, a positive result may also be seen with other conditions. Numerous D-dimer assays have been developed for human patients.[10] Most are based on monoclonal antibodies against **human D-dimer (hD-d)**, and they vary widely with respect to sensitivity. Some of these also are currently available in kit form, permitting the rapid detection of fibrin degradation in emergency situations.

Tests for FDPs and D-dimer have also been applied in ill dogs with thromboses.[4,6,11,a-c] Several D-dimer assays that use monoclonal antibodies to hD-d have been evaluated in healthy dogs and dogs with DIC, TED, and other illnesses.[6,11,a-c] However, the specific degree of cross-reactivity with **canine D-dimer (cD-d)** has not been validated because purified cD-d and monoclonal antibodies have not been available. Such blood D-dimer tests are generally designed for use at commercial veterinary laboratories rather than in private practices, which can result in undue delay of appropriate treatment of critically ill dogs. Therefore, a specific cD-d **point-of-care (POC)** test kit would be highly desirable. The primary purpose of the study reported here was to assess the clinical usefulness of a cD-d POC test kit that uses a monoclonal antibody to cD-d for samples obtained from dogs with DIC, acute TED, chronic TED, and hemorrhage. A secondary objective was to compare results of the cD-d POC test, a **latex agglutination (LA)** D-dimer assay, and an FDP assay with results of a quantitative ELISA that used the same monoclonal antibody to cD-d.

Materials and Methods

Animals—Dogs selected for use in the study were part of the population of dogs examined at the **Veterinary Hospital of the University of Pennsylvania (VHUP)**. The 71 client-owned dogs selected were prospectively assigned to 1 of 4 groups (12 healthy dogs, 18 dogs suspected of having DIC, 23 dogs with other TED, and 18 dogs with hemorrhage attributable to trauma or a hemostatic disorder other than DIC). Client consent was obtained from each owner prior to start of the study. The study reported here was approved by the Committee for the Use of Client-owned Animals in Research at VHUP.

The cD-d POC test[d] was performed, **prothrombin time (PT)** and **activated partial thromboplastin time (aPTT)** were determined, and a platelet count and evaluation for schistocytes were conducted on all dogs. Results for the cD-d ELISA,[e] an hD-d LA test[f] that has been evaluated in dogs,[6] and measurement of FDP concentrations were evaluated in samples obtained from 52, 43, and 68 of the 71 dogs, respectively (limited availability of plasma samples precluded performance of all tests for each dog). Other tests, including a CBC, serum biochemical analysis, urinalysis, microbial culture of a urine sample, radiography, ultrasonography, **computed tomography (CT)**, and determination of plasma **antithrombin III (AT III)** activity, were performed at the discretion of the primary clinician to identify underlying disease.

Dogs were deemed healthy when there was no history of bleeding or current medical problems, and results of physical examination and coagulation tests were within reference ranges. For the purpose of this study, inclusion criteria for the DIC group included a primary disease process associated with DIC (eg, neoplasia, sepsis, **immune-mediated hemolytic anemia [IMHA]**, pancreatitis, or heatstroke)[12] and at least 2 of the following laboratory abnormalities: times for PT, aPTT, or both that were > 25% prolonged, compared with results for a simultaneously evaluated control sample (ie, prior to anticoagulant treatment), detection of schistocytes, thrombocytopenia (reference range, 150,000 to 400,000 cells/µL), and decreased plasma AT III activity (reference range, 75 to 120% of the activity of pooled canine reference plasma). Increased plasma FDP concentrations were not considered as an inclusion criterion in an attempt to avoid bias for dogs with positive results for the D-dimer tests.

The TED group was classified into acute and chronic TED subgroups. Inclusion criteria for the acute TED subgroup were diagnosis of an underlying disorder associated with TED (eg, protein-losing nephropathy, naturally occurring hyperadrenocorticism, and administration of glucocorticoids for a minimum of 7 days)[13-15]; evidence of a thrombus on ultrasonographic examination, a CT scan, or during necropsy; and acute onset of clinical signs (< 3 days) associated with the thrombus. Also included in this group were dogs with suspected pulmonary thromboembolism that had low arterial oxygen concentration and no radiographic or cytologic evidence of infectious or inflammatory bronchiolar or alveolar disease and dogs with paresis and ischemia of the affected limb. Dogs that had documented thrombi for > 2 weeks but otherwise fulfilled inclusion criteria for the TED group were assigned to a chronic TED subgroup; the chronic TED subgroup was subsequently excluded from statistical analysis because of an insufficient number of dogs. Dogs were excluded from the TED groups when they had evidence of hemorrhage or coagulopathy prior to anticoagulant treatment.

Inclusion criteria for dogs in the hemorrhage group were acute bleeding in the preceding 1 to 4 days as a result of trauma (eg, long-bone fractures) or a hemostatic disorder other than DIC, such as hepatic disease, intoxication attributable to ingestion of an anticoagulant rodenticide, hereditary coagulopathy, and **immune-mediated thrombocytopenia (IMT)**. Disseminated intravascular coagulation was excluded on the basis that there were < 2 abnormalities in the coagulation tests or there was a lack of a disease process known to be associated with DIC. Thromboembolic disease was excluded on the basis of lack of evidence of thrombosis. Dogs with IMT were tested within 3 days of initiation of corticosteroid treatment.

Collection of blood samples—For the D-dimer and coagulation tests, blood samples (1.8 mL) were obtained with minimal trauma via venipuncture of a cephalic, saphenous, or jugular vein. Samples were collected directly into evacuated tubes containing 3.8% sodium citrate (0.2 mL) as the anticoagulant. Blood samples were immediately mixed with the anticoagulant and placed on ice, and plasma was separated by centrifugation within 30 minutes after collection. Plasma samples were analyzed immediately or stored frozen at –70°C for subsequent analysis. All samples were analyzed within 7 months after they were obtained. When needed, additional samples were obtained as part of the routine diagnostic evaluation of the dogs.

D-dimer assays—Three D-dimer assays were used. The cD-d POC test kit[d] included a test cartridge, a transfer pipette, and buffer solution, and the immunochromatography assay was performed in accordance with the manufacturer's instructions. Briefly, 1 drop of plasma was added to the sample well, which was followed by the addition of 2 drops of buffer. After a 5-minute incubation period during which the cartridge was on a flat surface at 20°C, the color change in the reading window of the kit was assessed by 1 of the investigators (AG). This person was aware of the clinical

condition of each of the dogs. Results for samples obtained from 52 dogs were also interpreted by personnel at the manufacturer, who were not aware of the clinical status or results of other diagnostic tests for the dogs.

This immunochromatography assay used sensitized colloidal-gold particles bound to a specific murine monoclonal antibody to detect cD-d antigen within plasma. As established for human kit assays, the antibody-gold conjugate binds specifically to D-dimer to form a complex, which is then captured on a sensitized reaction line. Accumulation of the complex causes formation of a pink-purple line. The appearance of a control line containing a sheep anti-mouse antibody that recognizes the mouse anti-D-dimer antibody-gold conjugate ensures that the test kit performed correctly (in the study reported here, this line was clearly visible for all samples).[d] Detection of any visible test line was interpreted as a positive result, and in most cases, strength of line color was subjectively assessed and recorded on a scale of 1 to 3, reflecting a weak, moderate, and strong reaction, respectively.

The cD-d ELISA[e] was a quantitative laboratory test based on the same monoclonal antibody to cD-d that was used in the cD-d POC test. The cD-ELISA was performed by personnel at a commercial laboratory in Australia[g]; personnel were not aware of the clinical status and cD-d POC results for the dogs. Samples were only evaluated when they were cold at the time of arrival at the laboratory after transportation to Australia. According to the manufacturer[g] of the ELISA, frozen samples are stable for at least 1 year when measured repeatedly. Because a purified cD-d was not available for use in the study, the data were expressed as the number of arbitrary units per milliliter. The lower limit of sensitivity of the ELISA was 1 U/mL, and values < 1 U/mL were expressed as 0 U/mL. Unfortunately, cD-d ELISA results were not available for all samples because 18 samples were damaged during transport to the laboratory in Australia.

Finally, the qualitative hD-d LA assay,[f] which has been evaluated for use in dogs,[6] was performed in accordance with the manufacturer's instructions by personnel at the clinical laboratory at VHUP; these personnel routinely use this plasma D-dimer assay, and they were not aware of the clinical status or other results for the dogs. The reference range for this assay was < 0.25 µg/mL, and positive results for the hD-d LA assay were reported only as > 0.25 µg/mL. This assay could also have been used to provide semiquantitative results by the use of serial dilutions; however, this option was not used for this study.

Other hemostatic assays—Platelet counts were performed by automated[h] or manual methods by use of EDTA-anticoagulated blood samples. Blood smears were examined to verify a decrease in platelet counts and to detect schistocytes. The coagulation variables PT and aPTT were measured in citrated plasma samples by use of conventional reagents[i,j] and a fibrometer.[k] Concentration of FDPs was measured in citrated plasma in accordance with the manufacturer's[l] recommendation by personnel at the VHUP clinical laboratory, with positive results reported as a value within the range of 5 to 20 µg/mL or a value of > 20 µg/mL (reference range, < 5 µg/mL). The activity of AT III was determined in citrated plasma by use of a chromogenic substrate kit,[m] with results reported as a percentage of the activity for pooled canine reference plasma (AT III activity of the pooled reference plasma was assigned a value of 100%). The personnel performing the aforementioned tests were unaware of the clinical status of the dogs.

Statistical analysis—An ANOVA was used to determine differences in cD-d ELISA measurements on the basis of group, results of cD-d POC and hD-d LA assays, and FDP concentration. Linear regression analysis was applied to evaluate the relationship between results for the cD-d ELISA and cD-d POC assay. Sensitivity, specificity, and positive- and negative-predictive values with 95% confidence intervals were calculated. A true-positive result was defined as a positive result for a dog with DIC, TED, or hemorrhage, and a false-negative result was defined as a negative result for a dog in any of these groups. A true-negative result was defined as a negative result for a healthy dog, and a false-positive result was defined as a positive result for a healthy dog. All analyses were performed by use of statistical software.[n] Results were reported as mean ± SD. Values were considered significantly different at $P < 0.05$.

Results

Healthy dogs—The 12 healthy dogs ranged from 2 to 11 years of age (mean, 4.8 years; median, 4 years) and included 10 males (1 sexually intact male and 9 neutered males) and 2 neutered females. There were 4 Labrador Retrievers, 2 mixed-breed dogs, 2 American Pit Bull Terriers, and 1 each of 4 other breeds. Analyzing the cD-d POC results for citrated plasma obtained from these healthy dogs revealed a single control line; discoloration was not evident at the site of the D-dimer test line. This indicated a negative result in all dogs. Results of the cD-d ELISA for these 12 healthy dogs were 0 or 1 U/mL (mean ± SD, 0.2 ± 0.4 U/mL). Results of the hD-d LA assay were negative for 9 of the 10 dogs tested, and FDP results were negative for all 12 dogs.

Dogs with DIC—Blood samples were collected from 18 systemically ill dogs suspected of having DIC on the basis of laboratory results and detection of an underlying disease associated with DIC. The dogs ranged from 1 to 12 years of age (mean, 6.9 years; median, 7 years) and included 8 males (1 sexually intact male and 7 neutered males) and 10 females (1 sexually intact female and 9 neutered females). There were 3 mixed-breed dogs, 2 Golden Retrievers, 2 Standard Poodles, and 1 each of 11 other breeds. Underlying diseases included pancreatitis (n = 5), neoplasia (4), septicemia (2), IMHA (2), acute liver failure (2; 1 attributable to leptospirosis and 1 of unknown cause), heatstroke (1), gastric dilatation and volvulus (1), and acute renal failure attributable to leptospirosis (1). Five dogs had evidence of hemorrhage (3 with hematemesis and 2 with multifocal hemorrhage detected during necropsy), and 2 dogs had evidence of microthrombi during necropsy.

Results for the cD-d POC test on plasma obtained from all 18 dogs revealed a pink-purple D-dimer test line in addition to the control line, indicating a positive result in all 18 dogs. In 14 of the 18 cD-d POC tests, the color change was subjectively assessed as moderate to strong. The cD-d ELISA was conducted for 9 of 18 samples, and results ranged from 6 to 67 U/mL (mean, 44 ± 23 U/mL). Only 1 sample (obtained from a dog with pancreatitis) had a value < 10 U/mL; this sample had positive results for the cD-d POC and hD-d LA assays and a negative result for the FDP assay. Values for the cD-d ELISA for dogs with DIC did not overlap with those of healthy dogs, and these values were significantly ($P < 0.001$) different from those of healthy dogs (**Fig 2**). Results of the hD-d LA assay were positive in 9 of 11 dogs tested. The 2 dogs with negative results for the hD-d LA assay had positive results for the cD-d

POC and FDP (> 20 µg/mL) tests, but values for the cD-d ELISA were not determined for either dog. Results for the FDP test were positive in 15 (2 dogs within the range of 5 to 20 µg/mL and 13 dogs with > 20 µg/mL) of 18 dogs. The 3 dogs with negative results for the FDP test had positive results for the cD-d POC and hD-d LA assays and had cD-d ELISA values > 23 U/mL.

Dogs with acute TED—The 19 dogs with acute TED ranged from 3 to 13 years of age (mean, 8.5 years; median, 8 years) and included 10 neutered males and 9 neutered females. There were 7 mixed-breed dogs, 5 Labrador Retrievers, 2 Cocker Spaniels, and 1 each of 5 other breeds. Underlying diseases for which immunosuppressive doses of glucocorticoids were part of the ongoing treatment included IMHA (n = 5), concurrent IMHA and IMT(3), IMT (2), chronic active hepatitis (1), and chronic allergic bronchitis (1). Two dogs were treated for 7 and 9 days, and all others for at least 14 days, with glucocorticoids. Other underlying conditions included naturally occurring hyperadrenocorticism (n = 2), neoplasia (2), diabetic ketoacidosis with systemic inflammatory response syndrome (1), total hip replacement with aspiration pneumonia (1), and protein-losing enteropathy (1). Vasculature occluded by thrombi included multiple vessels (n = 6) and the pulmonary artery (5), portal vein (3), aorta (1), splenic artery (1), jugular vein (1), caudal vena cava (1), and brachial artery (1).

Results for the cD-d POC test revealed that plasma from 10 dogs yielded a moderate to strong pink-purple D-dimer test line, whereas in 7 and 2 dogs, a weak test line or no test line was detected, respectively. Thus, 17 of 19 samples yielded a positive result. Results of the cD-d ELISA were available for 14 of 19 dogs, with values ranging from 8 to 66 U/mL (mean, 34 ± 19 U/mL). The cD-d ELISA values for dogs with acute TED were significantly ($P < 0.001$) higher than those of healthy dogs but were not significantly ($P = 0.16$) different from those of dogs with DIC (Fig 2). Results for the hD-d LA assay were positive for 10 of 12 dogs tested. Both dogs with negative results for the hD-d LA assay had positive results for the cD-d POC test. In addition, 1 of those dogs had a cD-d ELISA value of 27 U/mL but a negative result for the FDP test, whereas we did not determine a cD-d ELISA value for the other dog, but it did have a positive result for the FDP test (> 20 µg/mL). Results of the FDP test were positive in 15 (6 within the range of 5 to 20 µg/mL and 9 with > 20 µg/mL) of 17 dogs tested, and both dogs with negative results for the FDP test had positive results for the cD-d POC assay. Two dogs in this group that had a negative result for the cD-d POC assay had positive results for the hD-d LA and FDP tests; 1 dog had a cD-d ELISA value of 16 U/mL, and the other did not have a cD-d ELISA performed because the sample was damaged during transport.

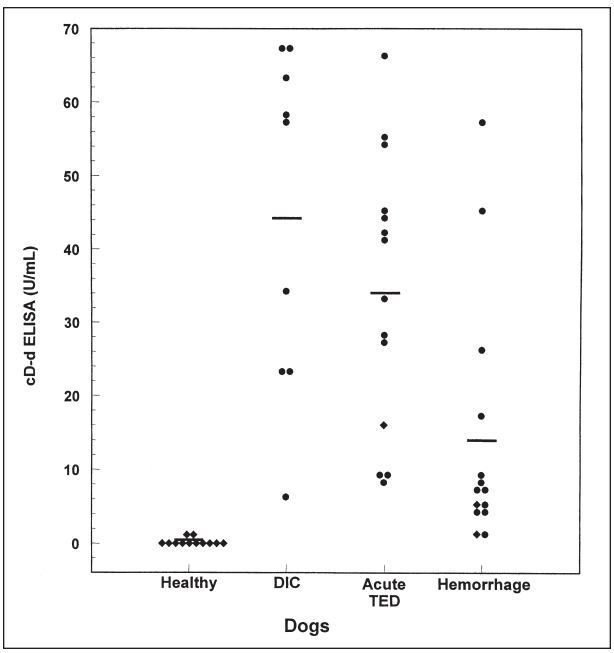

Figure 2—Values for a canine D-dimer (cD-d) ELISA on samples obtained from healthy dogs and dogs with disseminated intravascular coagulation (DIC), acute thromboembolic disease (TED), or hemorrhage. Each symbol represents 1 dog, and each horizontal bar represents mean ELISA values for a group. Dogs were also categorized on the basis of results for a cD-d point-of-care (POC) test (positive result, circle; negative result, diamond).

Dogs with chronic TED—The 4 dogs with chronic TED ranged from 5 to 11 years of age (mean, 8.2 years; median, 8 years) and included 1 sexually intact male and 3 neutered females, each of a separate breed. Underlying conditions included naturally occurring hyperadrenocorticism (n = 2) and conditions for which chronic immunosuppressive doses of glucocorticoids (> 14 days) were administered as treatment (IMHA [n = 1] and IMT [1]). Vasculature occluded by thrombi included multiple vessels (n = 1) and the pulmonary artery (1), portal vein (1), and caudal vena cava (1).

Use of the cD-d POC test revealed that plasma from 1 dog yielded a moderate pink-purple D-dimer test line, whereas in 1 and 2 dogs, a weak test line or no test line was detected, respectively. Thus, 2 of 4 samples yielded a positive result. Results of the cD-d ELISA were available for 3 of the 4 dogs, with values of 1, 5, and 11 U/mL (the latter 2 dogs had positive results for the cD-d POC test). Results of the hD-d LA assay were positive in 1 of 3 dogs tested, and results of FDP tests were positive (within the range of 5 to 20 µg/mL) in 1 of 4 dogs tested.

One dog with a thrombus in the portal vein was included in the acute TED subgroup and also subsequently included in the chronic TED subgroup, with an initial cD-d ELISA value of 44 U/mL that decreased to 9 U/mL 8 weeks later. Although results of the cD-d POC test were positive for both samples, the intensity of the line color decreased from moderate for the initial sample to weak for the second sample.

Dogs with hemorrhage—The 18 dogs with hemorrhage ranged from 4 months to 8 years of age (mean, 3.3 years; median, 2 years) and included 8 males (4 sexually intact males and 4 neutered males) and 10 females (3 sexually intact females and 7 neutered females). There were 2 mixed-breed dogs, 4 Labrador Retrievers, and 1 each of 12 other breeds. Reasons for hemorrhage included trauma (n = 8), IMT (3), intoxication attributable to ingestion of an anticoagulant rodenticide (3), chronic liver failure of unknown cause

(2), bleeding from a vaginal leiomyoma (1), and hemophilia A (1). The 3 dogs with IMT had PT and aPTT values within the reference ranges; were administered corticosteroid treatment for < 3 days; had platelet counts of 2,000, 6,000, and 18,000 cells/μL, respectively; and had no abnormalities consistent with thrombosis. One dog with coagulopathy attributable to chronic liver failure bled only after surgical biopsy. The D-dimer tests were performed 1 to 4 days after initial onset of bleeding in all dogs.

Results for the cD-d POC test revealed that plasma from 6 dogs yielded a moderate to strong D-dimer test line, whereas plasma from 9 dogs yielded a weak test line, and a test line was not visible for 3 samples. Thus, 15 of 18 samples had positive results for the cD-d POC test. Negative results for the cD-d POC test were for dogs with rodenticide intoxication (n = 2) and femoral fracture (1). Results of cD-d ELISA were available for 14 samples, with values ranging from 1 to 57 U/mL (mean, 14 ± 17 U/mL). The 2 dogs with negative results for the cD-d POC test that had cD-d ELISA values available had values of 1 and 5 U/mL. Values for the cD-d ELISA for dogs with hemorrhage were significantly lower than those for dogs with DIC ($P < 0.001$) and acute TED ($P = 0.002$) and significantly higher than those for healthy dogs ($P = 0.04$; Fig 2). Values of the cD-d ELISA were lowest for dogs with rodenticide intoxication (1, 4, and 5 U/mL, respectively), whereas dogs with hemorrhage for other reasons had highly variable cD-d ELISA values. Results for the hD-d LA assay were positive in all 8 dogs tested, and results for the FDP test were positive in 13 (6 within the range of 5 to 20 μg/mL and 7 > 20 μg/mL) of 17 dogs.

Comparison of results for the cD-d POC, hD-d LA, and FDP tests with results for the cD-d ELISA in healthy and clinically ill dogs—Overall, there was a significant ($P < 0.001$) difference between cD-d ELISA values for dogs with negative results for the cD-d POC test and dogs with positive results for the cD-d POC test (ELISA values of 1.6 ± 4.2 and 30.3 ± 22.3 U/mL, respectively; **Fig 3**). In addition, there was a significant ($P < 0.001$) difference between cD-d ELISA values of dogs with a weak positive result for the cD-d POC test (16.2 ± 16.8 U/mL), compared with ELISA values for dogs with a moderate (42.5 ± 17.3 U/mL) or strong (46.7 ± 21.9 U/mL) positive result for the cD-d POC test. However, there was considerable overlap of cD-d ELISA values among all dogs with positive results (weak, moderate, and strong) for the cD-d POC test. There was a strong positive correlation (R, 0.75) between intensity of color of the test line for the cD-d POC test and results for the cD-d ELISA. A positive result for the cD-d POC test was most likely when the cD-d ELISA value was ≥ 4 U/mL; for 38 of 43 (88%) samples with a cD-d ELISA value ≥ 4 U/mL, the cD-d POC test yielded a positive result, whereas for 16 of 17 (94%) samples with a cD-d ELISA value < 4 U/mL, the cD-d POC test yielded a negative result.

Results for the hD-d LA test were reported as negative (< 0.25 μg/mL) or positive (> 0.25 μg/mL). Mean cD-d ELISA value corresponding to the samples with negative results for the hD-d LA test (2.8 ± 8.5 U/mL) was significantly ($P < 0.001$) different from the mean value for samples that had positive results for the hD-d LA test (30.9 ± 21.9 U/mL; **Fig 4**). Similarly, dogs with FDP concentrations < 5 μg/mL (negative result) had significantly ($P < 0.001$) lower mean cD-d ELISA values (9.5 ± 19.6 U/mL) than dogs with FDP concentrations > 20 μg/mL (38.3 ± 22.4 U/mL; **Fig 5**). In addition, cD-d ELISA values for dogs with FDP concentrations > 20 μg/mL were significantly ($P = 0.03$) higher than values for dogs with FDP concentrations within the range of 5

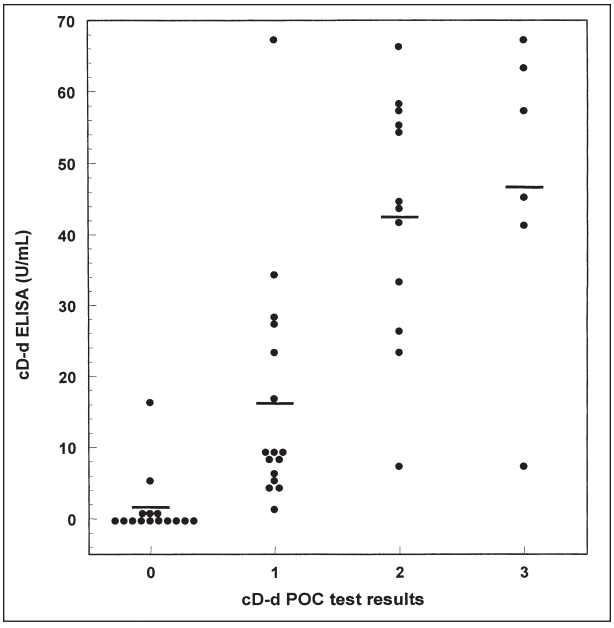

Figure 3—Values for a cD-d ELISA plotted against semi-quantitative results for a cD-d POC test. Results for the test line of the POC test were scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. Each symbol represents 1 dog, and each horizontal bar represents mean ELISA values for a group.

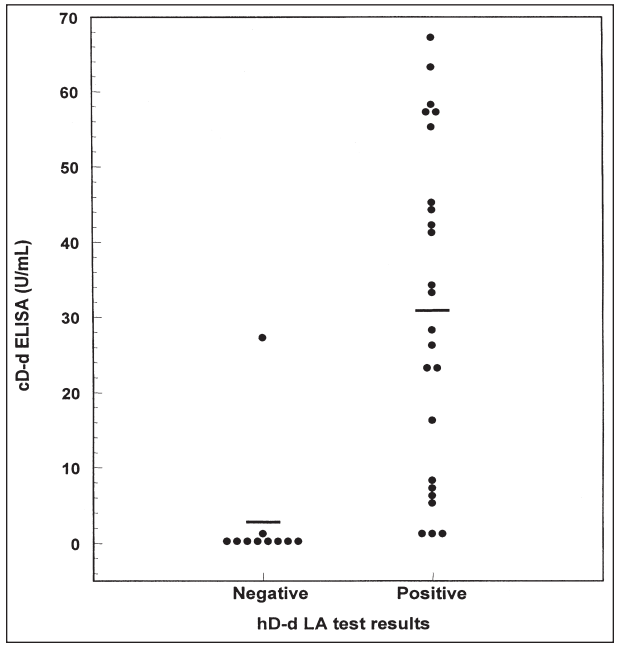

Figure 4—Values for a cD-d ELISA plotted against results for a human D-dimer (hD-d) latex agglutination (LA) test. Each symbol represents 1 dog, and each horizontal bar represents mean ELISA values for a group.

to 20 µg/mL (21.7 ± 16.1 U/mL). However, there was not a significant (P = 0.10) difference in mean cD-d ELISA values when comparing dogs with negative results for the FDP test with those that had FDP concentrations within the range of 5 to 20 µg/mL.

Sensitivity, specificity, and predictive values for the cD-d POC, hD-d LA, cD-d ELISA, and FDP tests—The cD-d POC and hD-d LA tests were comparable for use in differentiating healthy dogs from affected dogs (DIC, acute TED, and hemorrhage) and appeared to be superior to measurement of FDP concentrations (**Table 1**). When the cutoff value for a positive result for the cD-d ELISA was set at ≥ 2 U/mL (value chosen on the basis that all healthy dogs in this study had cD-d ELISA values < 2 U/mL), sensitivity and positive- and negative-predictive values of the cD-d ELISA were excellent.

Concordance of interpretation of results for the cD-d POC test between users—Interpretation of test results determined by the investigators and personnel at the manufacturer was in agreement for 47 of 52 (90%) dogs. There was disagreement only for samples in which the color intensity of the test line was subjectively assessed as 1 (weak reaction); for 4 of 5 dogs, (cD-d ELISA values of 1, 5, 5, and 16 U/mL, respectively), samples were interpreted as negative results by the authors and positive results by personnel at the manufacturer.

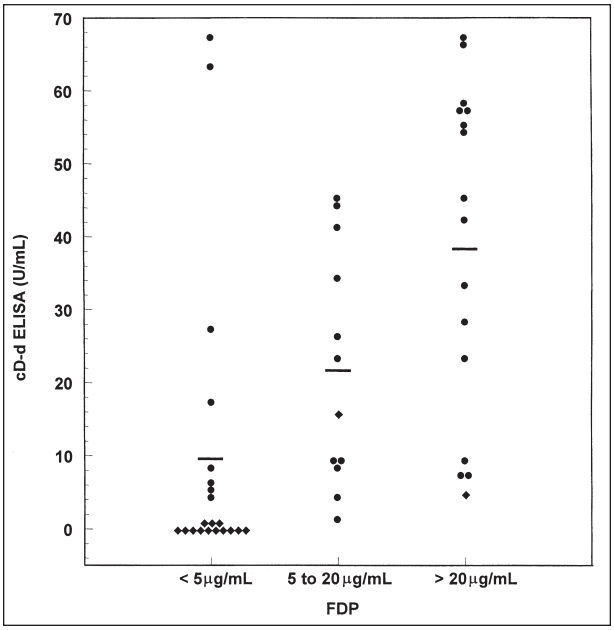

Figure 5—Values for a cD-d ELISA plotted against results for a fibrin degradation product (FDP) assay. Each symbol represents 1 dog, and each horizontal bar represents mean ELISA values for a group. Dogs were also categorized on the basis of results for a cD-d point-of-care (POC) test (positive result, circle; negative result, diamond).

Discussion

Disseminated intravascular coagulation and TED represent clinical emergencies that may pose a diagnostic challenge to small animal clinicians. It is difficult to quickly and specifically identify these thromboembolic complications. Therefore, a POC test that identifies fibrin formation and degradation for dogs would be highly advantageous. It appears that the cD-d POC test kit can be quickly and simply performed. It uses a small sample size (approx 25 µL of plasma), reliably detects dogs with DIC or acute TED, and compares well to other hD-d and FDP tests in dogs.[6,a-c] However, positive results for a D-dimer test may also be seen with other conditions in which clots form and undergo fibrinolysis, such as with any internal hemorrhage.

Three D-dimer assays (ie, the cD-d POC test, cD-d ELISA, and hD-d LA test) and the FDP assay performed well for samples obtained from healthy dogs, yielding negative results or low absolute values, except for 1 result for the hD-d LA test. These findings are consistent with results of studies in which investigators evaluated blood concentrations of D-dimer[6,a-c] and plasma concentrations of FDP[4,6,c] in healthy dogs.

Dogs with DIC had the highest mean cD-d ELISA value (44 ± 23 U/mL) among the affected dogs evaluated, which was significantly greater than that of healthy dogs (0.2 ± 0.4 U/mL) and dogs with hemorrhage (14 ± 17 U/mL). In addition, results of the cD-d POC test were positive in all dogs with DIC, suggesting that this assay may be slightly more sensitive than the hD-d LA (9/11 [82%] dogs) and FDP (15/18 [83%] dogs) assays for use in identifying dogs with DIC. In another study,[6] the same hD-d LA test had sensitivity of 100%, and the FDP test had sensitivity of 90%, for identifying 20 dogs with DIC. Whereas the specificity of each of the 4 assays performed in the study reported here was calculated to be between 90 and 100%, it must be recognized that this high specificity is attributable to comparison of affected dogs (DIC, TED, and hemorrhage) with a healthy population of dogs in which few, if any, positive results are expected. A positive result for the cD-d POC test is not diagnostic for DIC but is supportive of

Table 1—Sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value (NPV) for various tests used to differentiate healthy dogs from dogs with disseminated intravascular coagulation, acute thromboembolic disease, or hemorrhage

	Sensitivity		Specificity		PPV		NPV	
Test	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
cD-d POC	90.1	81.9 to 99.9	100.0	95.8 to 100	100.0	99.0 to 100	70.6	40.6 to 100
hD-d LA	87.1	72.6 to 100	90.0	64.8 to 100	96.4	87.6 to 100	69.2	33.5 to 100
FDP	78.2	64.7 to 91.7	100.0	95.8 to 100	100.0	98.8 to 100	50.0	17.5 to 82.5
cD-d ELISA*	94.6	85.7 to 100	100.0	95.8 to 100	100.0	98.6 to 100	85.7	61.7 to 100

*Determined by use of ≥ 2 U/mL as the cutoff value for a positive result.
95% CI = 95% Confidence interval. cD-d POC = Canine D-dimer point-of-care. hD-d LA = Human D-dimer latex agglutination. FDP = Fibrin degradation product. cD-d ELISA = Canine D-dimer ELISA.

the diagnosis in light of other clinical laboratory findings and the patient's underlying disease. However, a negative result for the cD-d POC test would make a diagnosis of DIC highly unlikely.

Similar to dogs with DIC, dogs with acute TED had a mean cD-d ELISA value (34 ± 19 U/mL) that was significantly higher than the value in healthy dogs or dogs with hemorrhage. Sensitivity of the cD-d POC test was 89%, identifying 17 of 19 dogs with acute TED. Sensitivity of the hD-d LA (12/14 [83%] dogs) and FDP (15/17 [88%] dogs) assays was < 100% for identifying dogs with acute TED in the study reported here. This sensitivity is slightly less than that reported in another study[a] in dogs in which a semiquantitative hD-d assay[o] had a sensitivity of 100% for detection of TED in 13 dogs. One of the 2 samples with a negative result for the cD-d POC test had a cD-d ELISA value of 16 U/mL; the other cD-d ELISA value was not determined because of damage to the sample during transport. Similar to the case for DIC, a positive result for the cD-d POC test supports a diagnosis of acute TED but is not specific for this hypercoagulable condition.

Negative results for the cD-d POC test and relatively low cD-d ELISA values may be expected in dogs with chronic TED because the half-life of D-dimers is approximately 5 hours in humans[16] and is likely to be similar in dogs. The D-dimer tests are used to monitor humans with TED because positive test results in patients with acute TED are expected to become negative over time if there is no active thrombosis. Changes in D-dimer and FDP concentrations following thrombus formation have not been reported in dogs and were not studied here; thus, their value in monitoring clinically ill dogs remains unknown.

Because dogs with internal hemorrhage will also have fibrin degradation, it is not unexpected to find positive results for FDP and D-dimer tests. Human trauma patients have positive results for D-dimer tests during the first 48 hours after injury.[17] Similarly, 15 of 18 (83%) dogs with hemorrhage had positive results for the cD-d POC test, and all 8 dogs tested had positive results for the hD-d LA test. Plasma D-dimer concentrations measured by use of the ELISA in dogs with hemorrhage were higher than D-dimer concentrations of healthy dogs but lower than values of dogs with DIC and acute TED. Presumably, intravascular fibrin degradation contributes to an increase in plasma D-dimer concentrations to a greater extent than does fibrin that is formed and degraded in cavities or with internal surface bleeding. The low D-dimer concentrations in dogs with intoxication attributable to ingestion of anticoagulant rodenticides may be explained by their inability to form fibrin clots. Furthermore, plasma FDP concentrations were increased in 13 of 17 (76%) dogs with hemorrhage, which may not be unexpected, because positive results for the FDP test also reflect degradation of fibrin. However, this finding is in contrast to that of an experimental study[18] in dogs in which investigators evaluated the effect of internal hemorrhage on FDP concentrations. In that study, increased concentrations of FDPs were not detected in any of the 7 dogs from 3 to 105 hours after the creation of hemoperitoneum or hematomas. The reason for this discrepancy is not evident because sensitivity of the FDP assays used in that study and the study reported here was similar.[4]

Overall, there was a significant relationship between the cD-d ELISA values and results for the cD-d POC test. In addition, there was a positive correlation (*R*, 0.75) between intensity of color of the test line for the cD-d POC test and the result for the cD-d ELISA; however, because of the substantial amount of variation in ELISA values within each group, it is not possible to conclude whether intensity of the test line is a clinically useful variable for differentiating among dogs with DIC, acute TED, or hemorrhage. Thus, a test line of any color intensity should be considered a positive result.

Discordance of 10% between the investigator and personnel at the manufacturer for interpretation of results of the cD-d POC test was attributable to differences in interpretation of samples with weak positive results. To minimize discordance, the manufacturer advises that all visible test lines be interpreted as a positive result.

Limitations of the study included the small number of dogs in each group, use of a selected population of dogs that is not representative of the general hospital population, a probable bias toward more severe cases of DIC and TED to meet inclusion criteria, and use of healthy dogs rather than clinically ill dogs that had conditions other than DIC or TED to provide data for calculating sensitivity and specificity of the assays. Follow-up studies to compare results for dogs with DIC or TED with results for dogs with similar underlying conditions but without DIC or TED will need to be performed to fully evaluate the clinical usefulness of the D-dimer tests. Disseminated intravascular coagulation and TED may be difficult to separate clinically, but a D-dimer test will not be used by practitioners to differentiate these groups, because test results would be expected to be positive for both groups. Two dogs with chronic liver disease were prospectively assigned to the hemorrhage group. Although it was not possible to exclude these dogs from the DIC group on the basis of laboratory data alone, chronic liver disease with coagulopathy is not considered to be a major underlying cause for DIC. On the basis of the encouraging results of this limited clinical study, additional studies to completely validate use of the cD-d POC test for dogs appear warranted.

Previously evaluated D-dimer assays have used monoclonal antibodies to hD-d,[6,11,a-c] whereas the POC test evaluated in the study reported here used a murine monoclonal antibody to cD-d and was the first assay developed specifically for use in dogs. The cD-d POC test was easy to perform, and it was sensitive but not specific for the diagnosis of DIC and TED because dogs with hemorrhage can also have positive results. This cD-d POC test compares favorably with other hD-d and FDP assays evaluated for use in dogs. The major benefit of the cD-d POC test kit may be the simplicity and rapidity of use in clinical practice. The cD-d POC test appears suitable for use in conjunction with other laboratory tests to aid in the diagnosis of DIC and acute TED in dogs

[a]Nelson OL, Andreasen C. The role of plasma D-dimer to detect thromboembolic disease in the dog (abstr). *J Vet Intern Med* 2002;16:375.
[b]Caldin M, Furlanello T, Berto D, et al. Preliminary investigations of

D-dimer concentrations in normal dogs and dogs with disseminated intravascular coagulation (DIC) (abstr). *J Vet Intern Med* 1997;11:130.

[c]Caldin M, Furlanello T, Lubas G. Sensitivity and specificity of citrated plasma FDPs and D-dimer in the diagnosis of disseminated intravascular coagulation (DIC) in dogs (abstr). *J Vet Intern Med* 1998;12:236.

[d]AGEN canine D-dimer, AGEN Biomedical Ltd, Brisbane, Australia.

[e]AGEN canine D-dimer enzyme immunoassay, AGEN Biomedical Ltd, Brisbane, Australia.

[f]D-dimer assay, Pacific Hemostasis, Humbersville, NC.

[g]AGEN Biomedical Ltd, Brisbane, Australia.

[h]Cell Dyn 3500 system, Abbott Diagnostics, Abbott Park, Ill.

[i]Thromboplastin liquid, Pacific Hemostasis, Huntersville, NC.

[j]Kontact, Pacific Hemostasis, Huntersville, NC.

[k]BBL fibrosystem precision coagulation timer, Becton-Dickinson Microbiology Systems, Cockeysville, Md.

[l]FDP plasma, Diagnostica Stago, American Bioproducts Co, Parsippany, NJ.

[m]Coatest antithrombin, diaPharma, West Chester, Ohio.

[n]SAS, version 8.0, SAS Institute Inc, Cary, NC.

[o]Semi-quantitative latex agglutination D-dimer assay, Synbiotics, San Diego, Calif.

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