Evaluation of a bench-top coagulation analyzer for measurement of prothrombin time, activated partial thromboplastin time, and fibrinogen concentrations in healthy dogs

Heather K. Mineo, DVM, and Rebecca B. Garabed, VMD, MPVM

Objective—To evaluate a bench-top coagulation analyzer for determination of prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration in healthy dogs.

Animals—55 healthy adult dogs.

Procedures—PT, APTT, and fibrinogen concentration were determined by use of the coagulation analyzer. Values were compared with results obtained independently by a conventional laboratory.

Results—Correlations (with 95% confidence intervals) between the coagulation analyzer and conventional laboratory values were 0.760 (0.610 to 0.857), 0.700 (0.448 to 0.721), and 0.896 (0.878 to 0.918) for PT, APTT, and fibrinogen concentration, respectively. Using linear regression, comparison of data from the coagulation analyzer and the conventional laboratory provided equations relating the coagulation analyzer values with values from the conventional laboratory and suggested that APTT and fibrinogen values from the coagulation analyzer and conventional laboratory were approximately the same within expected random variation. Prothrombin time values for the coagulation analyzer were significantly offset from the PT values for the conventional laboratory but still were correlated reasonably well with the conventional laboratory values.

Conclusions and Clinical Relevance—By use of the mechanical method of analysis, fibrinogen concentrations obtained with a bench-top coagulation analyzer correlated well with results for a conventional laboratory, indicating that the coagulation analyzer is a reliable instrument for determination of this coagulation variable. Coagulation analyzer results for PT and APTT correlated less strongly with those for the conventional laboratory, but they would still be considered clinically reliable. (Am J Vet Res 2007;68:1342–1347)

Patients in critical care settings often benefit from bedside or point-of-care diagnostic testing. Point-of-care testing is defined as any laboratory test performed outside a central laboratory by nonlaboratory personnel. Results that are reliable, accurate, and obtained quickly can help veterinarians determine the best course of treatment in what are often life-threatening situations. In human medicine, up to 39% of trauma patients have coagulopathies and require transfusions of blood or blood products. This is similar to veterinary medicine, and the incidence of coagulopathies, although not accurately determined, may be higher given the frequency of toxic events resulting from anticoagulants.

Studies in human medicine have revealed the benefits of point-of-care testing for reducing the duration of patient in-hospital stays and improving overall patient outcomes.

Patient care in critical care units differs from that in general practice and ward care. Life-threatening emergencies and subsequent complications arise frequently, and traditional laboratory testing often does not provide results in a sufficiently timely manner to enable appropriate treatment decisions to be made. Point-of-care testing is routinely performed and absolutely necessary in critical care settings to provide results within

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definition</th>
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<tr>
<td>PT</td>
<td>Prothrombin time</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated partial thrombin time</td>
</tr>
<tr>
<td>In</td>
<td>Natural logarithm</td>
</tr>
<tr>
<td>INR</td>
<td>International normalized ratio</td>
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<tr>
<td>ISI</td>
<td>International sensitivity index</td>
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a shorter period to minimize fatalities and improve patient outcomes.12 Physicians also have reported increased satisfaction with improved test turnaround time.

Many veterinary critical care facilities are equipped with routine near-patient diagnostic equipment, including serum biochemical, hematologic, electrolyte, and blood-gas analyzers. Complete evaluation of coagulation is still often performed at a traditional laboratory. Conventional test tubes and handheld analyzers are able to determine the activated coagulation time. Several point-of-care coagulation analyzers have been evaluated for use in human hospitals, and these results have been verified as accurate.13-17 Several bench-top veterinary coagulation analyzers are commercially available. To our knowledge, only one of them has been evaluated and validated to be accurate and reliable.18 Point-of-care tests for D-dimers19 and platelet function20 have been validated for use in dogs.

Primary and secondary hemostasis ensures adequate clot formation in healthy animals. Primary hemostasis refers to the formation of a stable platelet plug, whereas secondary hemostasis refers to stabilization of that plug by fibrin via activation of additional coagulation factors.21 Acquired and hereditary coagulopathies account for a substantial number of animals treated in veterinary critical care units. Accurate assessment of these coagulopathies is essential for the successful outcome of these animals. Coagulation evaluation involves measurement and interaction of procoagulant and anticoagulant factors and assessment of the number and function of platelets.

Coagulation status is evaluated via in vitro activation of specific parts of the coagulation cascade, which is followed by determining the time elapsed until clot formation.22-24 Prothrombin time is used to evaluate the activity of coagulation factor VII (traditionally referred to as the extrinsic coagulation pathway). Activated partial thrombin time is used to evaluate the activity of coagulation factors VIII, IX, XI, and XII (traditionally referred to as the intrinsic coagulation pathway). Both PT and aPTT evaluate the function of coagulation factors I, II, V, and X (traditionally referred to as the common coagulation pathway). Fibrinogen is cleaved by thrombin to form fibrin monomers that become cross-linked for clot stabilization. Measurement of D-dimers is used to evaluate cross-linked fibrin degradation products and to identify excessive fibrinolysis in pathologic states.

In the study reported here, a bench-top coagulation analyzer was evaluated to determine its accuracy and reliability for use in dogs. Results were compared against those for the same samples evaluated at a conventional laboratory. The objective of the study was to determine whether the bench-top coagulation analyzer would prove to be reliable as a point-of-care coagulation analyzer for use in veterinary settings.

Materials and Methods

Animals—Fifty-five healthy adult dogs were used in the study. Forty dogs were selected from a private veterinary blood bank organization, and 15 were from the patient population of a private veterinary emergency and critical care hospital. Dogs from the blood bank organization were all deemed to be healthy at the time of sample collection on the basis that they were not currently receiving any medications, had no chronic or acute medical conditions, and appeared to be in good health. The 15 other dogs had been referred to the private veterinary emergency and critical care hospital for various illnesses, and all were considered to have recovered from those conditions. They were examined at the hospital as part of a final evaluation and were considered to be healthy at the time of sample collection.

Collection of samples—A venous blood sample was obtained from each dog via jugular venipuncture. A 20-gauge needle was used for each venipuncture. Each sample was collected in entirety by use of a single clean-stick technique. Samples that required probing or multiple venipunctures for collection were excluded from the study.

Duplicate blood samples (1.8 mL/sample) were collected directly into each of 2 evacuated glass tubes that contained 0.2 mL of buffered 3.8% sodium citrate as an anticoagulant. The collection tubes were labeled and stored in a refrigerator for approximately 2 hours prior to processing. During sample processing, the tubes were centrifuged to promote separation of plasma from blood cells. Plasma was then harvested from each tube, placed into a plastic sample tube, and frozen. Samples remained frozen at approximately –17.8°C for 2 weeks before they were evaluated.

Procedures—For each paired sample of frozen plasma, 1 tube was analyzed by use of a bench-top coagulation analyzer and the other tube was submitted to a conventional laboratory for analysis. For the coagulation analyzer, samples were thawed and analyzed in accordance with the manufacturer’s instructions. Samples were analyzed by use of the analyzer’s mechanical and optical methods. Both methods of analysis were recommended by the manufacturer, although the reliability of either method has not been validated. Samples submitted to the conventional laboratory were thawed and evaluated by use of an automated coagulation analyzer via mechanical methods conducted in accordance with the manufacturer’s operating directives.

Mechanical measurement of clotting times relies on physical formation of fibrin strands that attach to a stainless-steel ball within the sample measuring well. Each measuring well has a cylinder that rotates under the amplitude and frequency of the ball’s movements. Formation of fibrin filaments changes the amplitude and frequency of the ball’s movements. When a stable clot is formed, movement of the ball ceases completely. The sensor registers the changes in the ball’s movement and automatically records the time interval.

Each assay for the bench-top coagulation analyzer was performed with the appropriate reagents or activators and in accordance with the manufacturer’s instructions. Tissue factor composed of lyophilized rabbit brain extract with buffers and stabilizers was used as the reagent for the PT assay. Calcium is the activator for the PT assay. A proprietary substance composed of puri-
fied rabbit brain cephalin in ellagic acid with buffers, stabilizers, and preservative was used as the reagent for the APTT assay. The activator for the APTT assay is calcium chloride. The fibrinogen assay was performed with lyophilized bovine thrombin reagent with buffers, stabilizers, and preservative. The conventional laboratory used rabbit brain–based tissue factor, the proprietary substance composed of rabbit brain cephalin in ellagic acid, and bovine thrombin as reagents for the PT, APTT, and fibrinogen assays.

Optical measurement of clotting times by the bench-top coagulation analyzer relied on diminished light transmittance that resulted from coagulation. The initial light transmittance of each sample was measured, and change in transmittance was continuously measured. Each sample was mixed with reagent and allocated to optical measuring wells. A light beam (405 nm) was passed through the wells and monitored by a sensor that recorded any change in transmittance of the reaction mixture. As coagulation progressed, the optical density of the sample-reagent mixture changed. When the coagulation reaction was complete, the time interval was recorded.

Measurement of fibrinogen concentration depended on the quantitative conversion of fibrinogen to fibrin. Thrombin was added to the citrated plasma sample, and the interval from the addition of thrombin to clot formation was termed the thrombin time. The rate of this quantitative conversion was measured by a method described elsewhere. Excess thrombin was combined with diluted plasma, which yielded a fibrinogen concentration inversely proportional to the clotting time. This curvilinear relationship was plotted and compared with a calibration curve prepared from a fibrinogen reference to determine the fibrinogen concentration in the sample. A new fibrinogen reference curve was generated for each lot of thrombin reagent and fibrinogen reference used in the bench-top coagulation analyzer to ensure properly calibrated results and to avoid variations in the calculated fibrinogen value. Prior to sample evaluation, a reference fibrinogen curve was prepared in accordance with the manufacturer's instructions; this curve was used in determining plasma fibrinogen concentrations for each dog. The conventional laboratory also generated a fibrinogen reference curve for use in calibrating results.

Statistical analysis—Results of the 3 tests (PT, APTT, and fibrinogen concentration) were analyzed separately. Correlations were used to quantify the agreement of values for the bench-top coagulation analyzer and conventional laboratory. Also, a linear regression model was used to describe relationships between results for the coagulation analyzer and conventional laboratory. Because the APTT and fibrinogen values did not appear to be normally distributed, a Spearman rank correlation with bootstrapped confidence intervals (rather than the Pearson correlation used for the PT data) was used for the APTT and fibrinogen tests. Linear regression calculations used the ln transformations of these test values. All calculations were performed by use of a statistical program.

Results

Some tests were not conducted on samples from all dogs because of loss of samples, mishandling of samples, or machine failure. Therefore, samples from 30, 48, and 37 dogs were used for analysis of the PT, APTT, and fibrinogen tests, respectively.

On the basis of reference ranges provided by the conventional laboratory, most dogs had values within the reference ranges for PT and APTT, whereas most dogs were classified as having fibrinogen concentrations below the reference range (Table 1). The low fibrinogen values were between 87 and 141 mg/dL. Two dogs had APTT values (45.7 and 28.8 seconds, respectively) that were above the reference range; however, fibrinogen concentrations for those 2 dogs (310 and 837 mg/dL, respectively) were within or above the reference range for fibrinogen. One dog had a low APTT value (9.1 seconds) but a fibrinogen concentration (310 mg/dL) that was within the reference range.

By use of data for the mechanical method of the bench-top coagulation analyzer for all dogs, correlations between test results (with 95% confidence intervals) for the coagulation analyzer and conventional laboratory were 0.760 (0.610 to 0.857), 0.700 (0.448 to 0.721), and 0.896 (0.878 to 0.918) for the PT, APTT, and fibrinogen assays, respectively. Correlation coefficients range from –1 to 1, with 0 indicating no correlation, 1 indicating perfect positive correlation, and –1 indicating perfect negative correlation. Different correlations were obtained when the results of the mechanical method for the bench-top coagulation analyzer were stratified for the 2 populations of dogs (ie, blood bank dogs and emergency clinic dogs) used in the study (Table 2). Use of the optical method for the bench-top coagulation analyzer resulted in lower correlations of 0.206 (–0.108 to 0.637), 0.433 (0.181 to 0.543), and 0.788 (0.729 to –0.837) for the PT, APTT, and fibrinogen assays, respectively.

Table 1—Classification of dogs on the basis of test results for a conventional laboratory.

<table>
<thead>
<tr>
<th>Classification</th>
<th>PT (6.0 to 12.0 s)</th>
<th>APTT (10.0 to 25.0 s)</th>
<th>Fibrinogen (150 to 400 mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 6.0 s</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Within 6.0 s</td>
<td>50</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>Above 25.0 s</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>48</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 2—Correlation (95% confidence interval) for test results determined by use of the bench-top coagulation analyzer and a conventional laboratory for 2 populations of healthy adult dogs.

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of dogs</th>
<th>PT</th>
<th>APTT</th>
<th>Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood bank dogs</td>
<td>40</td>
<td>0.555 (0.282–0.745)</td>
<td>0.585 (0.200–0.637)</td>
<td>0.769 (0.723–0.813)</td>
</tr>
<tr>
<td>Emergency clinic dogs</td>
<td>15</td>
<td>0.909 (0.717–0.973)</td>
<td>0.920 (0.670–0.956)</td>
<td>0.979 (0.952–1.000)</td>
</tr>
</tbody>
</table>
respectively. Linear regression models were constructed for comparing results for the bench-top coagulation analyzer with those for the conventional laboratory. Test results for the conventional laboratory were the dependent or response variable, and test results for the bench-top coagulation analyzer and population (blood bank or emergency clinic dogs) were the independent or predictor variables. Regression models were used to estimate equations that related values for the bench-top coagulation analyzer with those for the conventional laboratory. Test results for the conventional laboratory were the dependent or response variable (y), and test results for the bench-top coagulation analyzer (b) and population (blood bank or emergency clinic dogs) were the independent or predictor variables. The equation for the PT test was as follows: $y = 3.90 + (0.51 \times b) + C$. The equation for the APTT test was as follows: $ln y = -0.02 + (0.96 \times ln b) + C$. The equation for the fibrinogen test was as follows: $ln y = 0.19 + (1.00 \times ln b) + C$. In each equation, C represented a correction factor for emergency clinic dogs, compared with blood bank dogs. The value for C was $-0.26$, $-0.01$, and 0 for the PT, APTT, and fibrinogen tests, respectively.

The $R^2$ values for those equations were 0.589, 0.759, and 0.986 for the PT, APTT, and fibrinogen tests, respectively (1 extreme value was removed from the model for the fibrinogen concentration to improve the model fit). The $R^2$ value is often interpreted as the proportion of variation in the response (i.e., value measured by the conventional laboratory) that can be explained by the predictors (i.e., value measured by the bench-top coagulation analyzer and whether the dog was part of the blood bank or emergency clinic population). The estimated regression equations for the APTT and fibrinogen tests indicated that the bench-top and conventional-laboratory results were approximately the same within the expected random variation for the APTT and fibrinogen tests. However, PT results for the bench-top coagulation analyzer were significantly offset from values for the conventional laboratory (intercept, 3.90 seconds; $P < 0.001$ for the test of equality to 0). Once this offset was included, results from the bench-top coagulation analyzer were related to the value of the conventional world as indicated by a slope that was significantly ($P < 0.001$) different from 0. Thus, PT results for the bench-top coagulation analyzer related to those for the conventional laboratory but differed in scale. Thus, results for the bench-top coagulation analyzer were correlated with those for the conventional laboratory but differed slightly.

By use of the aforementioned equations, values were calculated for the bench-top coagulation analyzer that corresponded with cutoff values for reference ranges established by the conventional laboratory (Table 3).

The manufacturer of the bench-top coagulation analyzer independently calculated a reference range for each variable by use of a regression model based on the data from the blood bank population of dogs. To expand the number of dogs used to calculate a reference range, we included the emergency clinic dogs in our regression analysis. The calculated cutoff values from the regression model did not precisely match the cutoff values established by the manufacturer or the conventional laboratory.

Discussion

Overall, results for the bench-top coagulation analyzer were consistent with those for the conventional laboratory. Fibrinogen results correlated most strongly, whereas results for PT and APTT had less compelling correlations. Samples analyzed by use of the mechanical method for the bench-top coagulation analyzer provided results that correlated better with those from the conventional laboratory than those determined by use of the optical method for the bench-top coagulation analyzer. Therefore, we determined that analysis by use of the mechanical method for the bench-top coagulation analyzer provided more reliable and accurate results than for the optical method, compared with values from the conventional laboratory.

The 2 testing systems (bench-top coagulation analyzer and conventional laboratory) appeared to have differing correlations, depending on the study population tested. Overall, test results were more correlated for emergency clinic dogs as opposed to blood bank dogs, which may have been a result of dogs with higher values for all 3 tests being represented in the emergency clinic population. Linear regression of the PT results that used data only for the emergency clinic dogs suggested that values for the bench-top coagulation analyzer and conventional laboratory were more closely correlated in dogs from the emergency clinic ($R^2 = 0.826$, intercept = 1.88, and slope = 0.72); however, results of the linear regression model based on data from only emergency clinic dogs were highly influenced by 2 dogs with PT values $> 12.0$ seconds. Sensitivity of PT correlation results to outliers in the emergency clinic population suggested that the emergency clinic dogs were sufficiently different from the other population of dogs and should be included in future studies that evaluate coagulation tests, and results of the bench-top coagulation analyzer should not be assumed to correlate with results of the conventional laboratory without the inclusion of a more diverse population of dogs.

Reference ranges determined by the manufacturer of the bench-top coagulation analyzer varied slightly from those established by the conventional laboratory even when they were scaled by use of linear regression equations. This is to be expected because of differences in measurement methods and equipment. To address the issue of varying results attributable to differences in reagents, the International Committee for Standardization in Hematology and the International Committee

<table>
<thead>
<tr>
<th>Test</th>
<th>Conventional laboratory</th>
<th>Bench-top coagulation analyzer*</th>
<th>Manufacturer of the bench-top coagulation analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (s)</td>
<td>6.0–12.0</td>
<td>4.6–16.4</td>
<td>6.7–11.4</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>10.0–25.0</td>
<td>11.4–29.5</td>
<td>11.9–20.0</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>150–400</td>
<td>124–331</td>
<td>150–400</td>
</tr>
</tbody>
</table>

*Represents the reference range predicted by use of linear regression equations on the reference ranges for the conventional laboratory.
on Thrombosis and Hemostasis created an INR for reported PT values. The thromboplastin reagent used in a PT assay is assigned an ISI value after being calibrated against an international reference preparation with a defined ISI. The INR value is then calculated from the patient PT results, mean reference range PT, and thromboplastin ISI. Use of the ISI-IRN system allows for PT results to be determined independent of any specific thromboplastin reagent as though the sample from the patient had been tested directly against the international reference preparation.

Theoretically, had the thromboplastin reagents used by the bench-top coagulation analyzer or the conventional laboratory in the study reported here both been inappropriately calibrated against the international reference preparation, we could have expected substantial deviations in our results. However, the bench-top coagulation analyzer and conventional laboratory used the same thromboplastin reagent, so there was no difference in the ISI. Therefore, the calculated INR for each PT result was not expected to differ and cannot be considered a contributor to the variation in our results. It should be mentioned that no abnormal dogs were included in the study, so the calculated range should not be interpreted as a universal reference range but simply as a comparison of the values for the conventional laboratory and bench-top coagulation analyzer and how they relate to the recommended range for the bench-top coagulation analyzer.

Point-of-care testing has become established as a vital tool in the management of critically ill patients. This is especially true for the evaluation of hemostasis. However, the usefulness of these techniques is somewhat hampered by the need for intense performance assessment for the equipment and reagents involved. The manufacturer of the bench-top coagulation analyzer recommends that quality-control evaluation of the testing equipment and reagents be performed multiple times each day.27 This evaluation is recommended to account for day-to-day variation in performance by the point-of-care testing equipment. In addition, the assay reagents and activators are subjected to performance analysis and correlation studies by the manufacturer prior to distribution for use at private practices.26,27

Erratic or erroneous values, equipment malfunc tion, or both may result when appropriate quality-control measures are not maintained. Values may also be unreliable because of operator errors, such as sample mishandling, or inadequate quality assessment, such as failing to validate reagents. Although we performed quality-control and quality-assessment procedures in accordance with recommendations made by the manufacturer, an error in this area may have accounted for the significant differences in results for the bench-top coagulation analyzer, compared with those for the conventional laboratory. In fact, this same rule holds true for all laboratories in that quality-control and quality-assessment protocols must be followed to ensure accurate results.

Point-of-care diagnostic testing can benefit patients in critical care settings. Point-of-care laboratory analyzers are fairly standard in veterinary medicine and are required for veterinary emergency and critical care facilities. Animals with coagulopathies are commonly found in critical care settings, and there is a need for reliable, point-of-care coagulation analysis. The bench-top coagulation analyzer tested in the study reported here was a reliable instrument for use in dogs; thus, it should provide a viable option for in-hospital coagulation analysis in veterinary settings.

References

27. AMAX Alexin [package insert]. St Louis: Trinity Biotech USA, Year.
28. AMAX Fibrinogen [package insert]. St Louis: Trinity Biotech USA, Year.