Effect of storage conditions on hemostatic parameters of canine plasma obtained for transfusion

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Objective—To evaluate the effects of various storage conditions on one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), and fibrinogen concentration of canine plasma collected for transfusion.

Sample Population—Plasma from 9 dogs.

Procedure—Whole blood was collected from dogs by means of jugular venipuncture and centrifuged at 7,300 × g for 20 minutes at 0°C. A plasma extractor was then used to generate plasma. Aliquots of plasma were collected in segments of plastic tubing and in microcentrifuge tubes, and plasma collection bags, tubing segments, and microcentrifuge tubes were immediately frozen at −30°C. Additional tubing segments and microcentrifuge tubes were stored at 2°C. After 1 week of storage, all samples were thawed, and OSPT, APTT, and fibrinogen concentration were measured. Collection bags and microcentrifuge tubes were refrozen at −30°C, and values were measured again 30 days after blood collection.

Results—Values for OSPT, APTT, and fibrinogen concentration did not vary significantly with storage time, temperature, or container.

Conclusions and Clinical Relevance—Results suggested that storage for up to 30 days at 2°C versus −30°C did not have any significant effect on hemostatic parameters of canine plasma obtained for transfusion. (Am J Vet Res 2001;62:734–735)

Whole fresh blood and blood products (fresh plasma, fresh-frozen plasma, frozen plasma, platelet concentrates, and cryoprecipitate) are frequently used to treat bleeding disorders in dogs and cats in clinical practice. Canine plasma appears to be hemostatically stable when stored frozen, as previous studies1–3 did not reveal any significant changes in one-stage prothrombin time (OSPT) or activated partial thromboplastin time (APTT), compared with baseline values, for canine plasma stored for up to 7 days at −80°C. In those studies, however, plasma was obtained after blood was collected with a needle and syringe into tubes containing 3.8% trisodium citrate and was stored at −80°C. These conditions are quite different from those for plasma intended for transfusion. Plasma used for transfusion is typically obtained from blood collected, using a closed system, into bags containing citrate phosphate-double dextrose solution, and the plasma is typically stored in plastic bags at −30°C. In addition, the APTT of canine plasma collected in 3.8% trisodium citrate and stored for 370 days at −80°C was significantly shorter than baseline APTT and APTT measured after 1 week of storage,4 possibly because of release of tissue thromboplastin from WBC and platelets that caused continuous activation of the intrinsic coagulation system.

The purpose of the study reported here, therefore, was to evaluate the effects of various storage conditions on OSPT, APTT, and fibrinogen concentration in canine plasma collected for transfusion. Specifically, we evaluated the effects of storage for 1 week at 2°C, 1 week at −30°C, and 30 days at −30°C.

Materials and Methods

Whole blood was collected from 9 healthy mature dogs (mean age, 3.9 years; range, 3 to 8 years) enrolled in the blood donor program at The Ohio State University Veterinary Teaching Hospital. For all dogs, results of CBC and serum biochemical analyses were within reference limits; all dogs were seronegative for Dirofilaria immitis, Babesia canis, and Ehrlichia canis. A commercially available blood typing system5 was used to determine whether dogs were positive or negative for DEA 1.1. Four dogs were positive for DEA 1.1, and 5 were negative; the 5 dogs that were negative for DEA 1.1 were also negative for DEA 1.2 and 7. Prior to sample collection, PCV, serum urea nitrogen concentration, temperature, pulse rate, heart rate, respiratory rate, and activated coagulation time (ACT)6 were measured.

One unit (450 ml) of whole blood was collected in standard fashion7 from each dog by means of jugular venipuncture, using a 16-gauge needle attached to a quadruple-bag closed-collection system8 on a blood mixer.9 The closed-collection system consisted of a primary bag that contained 63 ml of citrate phosphate-double dextrose solution as an anticoagulant, an additive bag that contained 100 ml of additive solution,10 and 2 empty satellite bags.

Bags with whole fresh blood were centrifuged at 7,300 × g for 20 minutes at 0°C.1 A plasma extractor was then used to generate 2 bags of plasma from each unit of blood: 1 bag of plasma (20 to 30 ml) was used for the present study, and the other bag (approx 200 ml) was submitted to the Transfusion Medicine Service and used for routine transfusion of clinical patients at the veterinary teaching hospital. Both bags of plasma were immediately frozen at −30°C.

Small hand sealer clips11 were used to create 2 segments of tubing that each contained approximately 1 ml of plasma for each satellite bag. One segment was stored at 2°C, the other was stored at −30°C. Plasma from each dog was also collected in three 1.5-ml polypropylene microcentrifuge tubes.12 One
Table 1—Effects of storage conditions and storage container on hemostatic parameters of canine plasma obtained for transfusion function in dogs.1,5-7 In those studies, however, blood and storage on results of routine assays of hemostatic function were determined, using an automated analyzer and commercially available reagents.1 Samples in the bags and the tubes were frozen again at –30 C and analyzed in a similar fashion 30 days after collection.

Data were analyzed by use of repeated-measures ANOVA to compare storage container (bag, tubing segment, microcentrifuge tube), time of storage (1 week vs 30 days), and storage temperature (2 C vs –30 C). For all analyses, values of P < 0.05 were considered significant.

Results

Storage time, temperature, and container did not have any significant effects on values for OSPT, APTT, and fibrinogen concentration (Table 1). We did not detect any significant differences among mean values for samples.

Discussion

Results of the present study suggest that storage for up to 30 days and at 2 C versus –30 C did not have any significant effect on hemostatic parameters (OSPT, APTT, and fibrinogen concentration) of canine plasma obtained for transfusion. Consequently, results of hemostasis tests on plasma collected for transfusion and stored for up to 30 days at < 2 C should reflect those of the donor being evaluated.

Results of the present study are similar to results of previous studies that evaluated the effects of freezing and storage on results of routine assays of hemostatic function in dogs.1,5-7 In those studies, however, blood samples were collected by means of direct venipuncture, using a syringe and needle, and placed in glass or plastic tubes containing 3.2 or 3.8% sodium citrate before they were centrifuged, which does not mimic the conditions used for obtaining and storing plasma meant for transfusion. O’Neill et al11 evaluated hemostatic function in human plasma collected for transfusion and stored at various temperatures. They did not find any significant changes in factor V, VII, or X activities or in fibrinogen, antithrombin III, protein C, or protein S concentrations following storage at 4 or 22 C; however, they only examined the effects of storage for 24 hours.

In this study, we also determined whether the storage container (ie, the original blood collection bag, a segment of plastic tubing, or a microcentrifuge tube) had an effect on hemostatic parameters of canine plasma. When performing research on hemostatic function of canine plasma, it would be useful to be able to analyze multiple aliquots of a single sample at various times after collection. However, storage of plasma in plastic bags makes this impractical, inconvenient, and costly, because the entire sample has to be used immediately after thawing or discarded after analysis. Results of the present study suggest that aliquots of plasma samples can be stored frozen in segments of plastic tubing or in microcentrifuge tubes without affecting results of subsequent hemostatic analyses.

References