

EFFECT OF MANUALLY PREHEPARINIZED SYRINGES ON PACKED CELL VOLUME AND TOTAL SOLIDS IN BLOOD SAMPLES COLLECTED FROM AMERICAN ALLIGATORS (*ALLIGATOR MISSISSIPPIENSIS*)

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Abstract

The hemodilution effect of manually preheparinized syringes was determined by evaluating the packed cell volume (PCV) and total solids (TS) from blood samples collected from 50 American alligators (*Alligator mississippiensis*). A volume of 0.2 mL of blood was drawn into 3 syringes of 1 mL: control with no heparin sodium, heparin sodium drawn to 0.1 mL and then expelled, and heparin sodium drawn to 0.2 mL and then expelled. PCV and TS values were determined from each syringe immediately after collection. Mean PCV and TS values were compared between the control and heparin groups using linear mixed modeling. The syringes coated with heparin resulted in a significantly lower mean PCV and TS values when compared with controls, with no significant difference between the heparin groups. This dilution effect was also found to be inconsistent and not accurate from one syringe to another. An adjunct method of obtaining 0.5 mL from already-collected blood samples into 1-mL syringes that were coated with heparin drawn to 0.2 mL and then expelled also showed a significant decrease in PCV and TS values when compared with the control samples, although to a lesser extent. As a result, it cannot be recommended to manually preheparinize syringes when collecting small volume of blood samples from American alligators because significant and unpredictable hemodilution is likely to occur. Copyright 2014 Elsevier Inc. All rights reserved.

Key words: *Alligator mississippiensis*; American alligator; heparin; packed cell volume; syringe; total solids

Venipuncture in some exotic animal species can be challenging because of small, collapsible veins or the limitation of collecting a maximum volume of 1% of the animal's body weight in blood.¹ Blood collection and analysis for values contained in a complete blood count (CBC) and plasma/serum biochemical profile, as well as more advanced tests, are hallmarks of veterinary practice in evaluating the response and effect of diseases and monitoring health in animals. When only small volumes of blood can be collected, performing basic testing (e.g., packed cell volume [PCV] and total solids [TS]) is necessary to aid in diagnosing disease or as part of a preventative health screening.

It is a known and nonstandardized practice by some veterinarians in zoological medicine to manually heparinize syringes before collecting blood from certain species^{2,3} to decrease the risk of

clot formation. Increased risk of coagulation and fibrin clot formation in the syringe may occur in such cases where blood flow into the syringe is slow, as may be the case in patients with very small

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collapsible veins, low blood pressure due to slower metabolism, hypovolemia, and/or hypothermia.³ Moreover, repeated sampling may cause trauma to veins, which can increase the levels of tissue factors that promote coagulation. In addition, rabbit blood clots faster at room temperature when compared with other exotic mammal species,² and, in the authors' experience, blood from ratite and corvid species clots subjectively faster when compared with other avian species. Heparin acts by binding and accelerating the effects of antithrombin III⁴ and is considered an acceptable anticoagulant for avian, amphibian, reptilian, and small exotic mammal species.^{1,5-7} In some instances, heparin is drawn into the syringe, to coat the inside of the syringe, and is then expelled. As a result, residual heparin is contained in the needle hub and syringe dead space, in addition to a possible heparin film lining the inside of the syringe. A minimum of 0.2 to 0.3 mL of blood is required by a number of veterinary diagnostic laboratories to obtain a CBC or plasma/serum biochemical profile. These samples could be divided into 0.1 mL of blood plus slides for a CBC and 0.05 to 0.075 mL of plasma/serum for a biochemical profile. These small volumes may be more susceptible to dilution effects from the residual heparin left in a preheparinized syringe than a larger volume of blood.¹

Blood sample dilution due to heparin has been shown to cause preanalytical error related to PCO₂, PO₂, electrolytes, and lactate level measurements in domestic dogs, with ionized calcium being most affected because of heparin's ability to chelate calcium and other divalent cations.⁸ Although adding heparin to a syringe has been hypothesized to affect PCV and TS values,¹ there are no reports evaluating dilution effects of manually preheparinized syringes on PCV and TS values in any species. The objective of this study was to determine the hemodilution effect of manually preheparinized syringes by measuring PCV and TS of blood samples collected from American alligators (*Alligator mississippiensis*). Alligators were selected as the animal model for this study because of their availability and ease of venipuncture.

MATERIALS AND METHODS

Animals

This study was performed according to an established protocol approved by the Institutional Animal Care and Use Committee at Louisiana State University. A total of 50 American alligators that

were 2 years old from a single alligator farm in Covington, LA USA, were used for this study. The study was performed on the site at a single pen so that the animals were all exposed to the same husbandry conditions. Animals were fed a dry, 45% protein content, commercial alligator ration, and the water temperature was maintained at an average of 85°F (29.4°C).

Sample Collection

For blood collection, each animal was manually restrained, and approximately 5 mL of blood was collected from the lateral occipital sinus using a 20-gauge, 1.5-in blood-collection needle (Tyco Healthcare Group, Mansfield, MA USA) and stored in a 10-mL red-top serum tube (Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ USA). This single tube served as the common sample for each animal so as to avoid repeated venipuncture of the same animal and any variability that might arise from the venipuncture technique itself. Immediately after collection, 0.2 mL of blood was collected from the red-top tube into each of the 3 syringes of 1 mL (groups C, L, and H) fitted with a 25-gauge, 5/8-in hypodermic needle (Tyco Healthcare Group). The control group syringe (C) contained no heparin, one syringe was preheparinized with the plunger drawn to 0.1 mL and then expelled (L), and one syringe was preheparinized with the plunger drawn to 0.2 mL and then expelled (H), ensuring all syringes and needle hubs were free of air after drawing the heparin. The syringes were prepared less than 24 hours before blood collection using heparin sodium injection (1000 USP/mL, Sagent Pharmaceuticals, Schaumburg, IL USA). The 3 syringe groups were color coded, but investigators were blinded to the heparin content during the study. To prevent an order effect, the order of collection into each of the 3 syringes was randomized by animal using statistical software R (R development core team [2012] R foundation for statistical computing, Vienna, Austria; <http://www.R-project.org/>). After blood was collected into the 1-mL syringes, the common 10-mL red-top serum tube was inspected to ensure that no clots had formed in the sample obtained from each animal.

To determine PCV and TS values, a microhematocrit tube without anticoagulant (Chase Scientific Glass, Rockwood, TN USA) was filled from each of the 1-mL syringes and was then centrifuged for 5 minutes at 13,200g. The PCV was read to the nearest percentage using the microhematocrit medical record. The tubes were

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