Effect of the size of evacuated blood collection tubes on total carbon dioxide concentration in equine plasma

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Objective—To determine whether plasma total CO₂ concentrations would vary with the size of the evacuated tube used to collect blood samples.

Design—Randomized crossover study.

Animals—Convenience sample of 20 healthy adult horses.

Procedures—Jugular venous blood was collected from horses in random order into 8 types of evacuated tubes: 2-mL glass, 2- or 3-mL plastic or plastic plasma separator, 4- or 6-mL plastic, and 10-mL glass or plastic. Total CO₂ concentrations in plasma were measured with a biochemistry analyzer. Data were analyzed via repeated-measures ANOVA and multivariate regression.

Results—The air volume–to–blood volume ratio was significantly higher and consequently, plasma total CO₂ concentration was significantly lower when blood was collected into 2-mL glass tubes and 2- or 3-mL plastic tubes than when the other 5 types of evacuated tubes were used. Concentrations in the other tube types were statistically equivalent. A linear relationship was detected between total CO₂ concentration and air volume–to–blood volume ratio.

Conclusions and Clinical Relevance—Blood samples should be collected into evacuated tubes with a small air volume–to–blood volume ratio whenever an accurate estimate of plasma total CO₂ concentration is required. (J Am Vet Med Assoc 2012;241:922–926)

Glass or plastic evacuated tubes are commonly used in veterinary medicine to collect blood samples for plasma or serum biochemical analysis. Still and Rodman1 were the first to recognize the importance of using “fresh tubes with good vacuums, and to fill the tube to capacity, to avoid diffusion of CO₂ out of blood” when collecting blood into an evacuated tube. Underfilling of evacuated tubes reportedly results in a lower measured total CO₂ concentration than when tubes are fully filled because CO₂ is released into the air space above the blood.2–5 The loss of CO₂ results in a decrease in PCO₂ in plasma or serum after sample collection and thereby a decrease in plasma or serum total CO₂ concentration.1–4 This decrease in PCO₂ and plasma or serum total CO₂ concentration is related to the ratio of blood sample volume to air volume.6 An unrecognized but potentially important problem is that completely filled evacuated blood collection tubes of various sizes differ in their air volume–to–blood volume ratio. Although tube size has been suggested to be one of many factors that may contribute to a decrease in measured total CO₂ concentrations in plasma or serum during handling and storage,7 we are unaware of any published data regarding the relationship between evacuated tube size and measured total CO₂ concentration in appropriately filled evacuated tubes.

Preanalytic errors in the measurement of total CO₂ concentration may result in an incorrect interpretation of acid-base status and affect the ability to detect whether alkalining agents that may impact athletic performance have been administered to horses before a race.8 Therefore, we hypothesized that measured total CO₂ concentration would depend on the size of the evacuated tube used to collect blood because of tube differences in the air volume–to–blood volume ratio after collection. The objectives of the study reported here were to calculate the air volume–to–blood volume ratios of 8 commercially available evacuated tubes and to determine the effect of evacuated tube size and material (plastic or glass) on total CO₂ concentration in equine plasma.

Materials and Methods

Animals—Twenty healthy adult horses (6 Quarter Horses, 4 Standardbreds, 4 Thoroughbreds, 1 Arabian, 1 Paint, 1 Appaloosa, 1 Haflinger, 1 Tennessee Walking Horse, and 1 Saddlebred; 10 mares and 10 geldings; age, 6 to 23 years) were obtained from the Purdue University Department of Veterinary Clinical Sciences teaching herd. The horses were housed in a paddock and maintained on pasture with ad libitum access to water. Supplemental feed concentrate was fed as needed to any horse that was not maintaining its body weight.

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weight through feeding on pasture alone. All horses were deemed healthy on the basis of physical examination findings, and no clinical signs of disease had been observed within 3 months prior to the study. The study protocol was reviewed and approved by the Purdue University Animal Care and Use Committee.

**Blood sample collection**—The venipuncture site over the left jugular vein of each horse was cleaned with a 70% alcohol swab, and venous blood samples were collected into various tubes in random order (assigned by means of a random number generator) with a 20-gauge, 1-inch needle, and evacuated tube holder. The 8 types of evacuated tubes used were of various sizes and materials (plastic or glass) containing lithium heparin or sodium heparin as an anticoagulant. Specific tubes used were as follows: 2-mL glass tube containing sodium heparin, 2-mL plastic tube containing lithium heparin, 3-mL plastic tube containing lithium heparin, 3-mL plastic plasma separator tube containing lithium heparin, 4-mL plastic tube containing lithium heparin, 6-mL plastic tube containing lithium heparin, 10-mL glass tube containing sodium heparin, and 10-mL plastic tube containing lithium heparin.

**Blood sample collection** continued until blood stopped flowing, and great care was taken to ensure atmospheric air was not aspirated into the tube during the collection process. All tubes were gently inverted 8 to 10 times immediately after sample collection to appropriately mix the blood and anticoagulant in accordance with the manufacturer’s recommendations. Blood samples were then stored upright in an insulated cooler with an internal temperature of approximately 4°C and transported to the laboratory. Blood samples were collected and analyzed from 1 to 2 horses each day.

**Determination of air volume and blood volume in evacuated tubes**—The height of blood within the filled evacuated tube was measured to permit calculation of the air volume and blood volume through use of the internal dimensions of the tube and rubber cap and the following formula for the volume of a cylinder:

\[ \text{Volume} = \pi \times r^2 \times h \]

in which \( r \) = radius and \( h \) = height. Beginning with the last 10 horses, each evacuated tube was weighed and the weight of blood in the tube was calculated. All tubes were centrifuged for 15 minutes at 1,000 \( \times \) g in a fixed-angle centrifuge as recommended by the tube manufacturer within 30 minutes after collection. All samples were analyzed within 1 hour after centrifugation.

**Determination of pressure in the evacuated tube**—A 1-inch, 16-gauge needle was attached to a pressure transducer. The output signal from the pressure transducer was recorded with the aid of computer software. The pressure transducer was zeroed to atmospheric pressure and calibrated with a water manometer. The pressure inside each evacuated tube was determined by quickly inserting the needle into the tube through the cap, with the tube held in a horizontal position. Five to 10 tubes from the same lot number representing each of the following tube types were used to obtain pressure measurements: 6-mL plastic, 4-mL plastic, 3-mL plastic plasma separator, 3-mL plastic, 2-mL glass, and 2-mL plastic tubes. Pressure could not be accurately measured in the 10-mL plastic and 10-mL glass tubes because the pressure exceeded the dynamic range of the transducer.

**Measurement of plasma total CO concentration**—A total CO analyzer was maintained and calibrated in accordance with the manufacturer’s recommendations with the supplied high (30 mmol/L) and low (0 mmol/L) total CO concentration calibrators. Aqueous total CO standards (5, 10, 20, 30, and 40 mmol/L) were used to confirm linearity of measurements made by the total CO analyzer (\( R^2 = 0.999 \) to 1.000 for 20 analytic runs). Combined interassay and intra-assay mean ± SD (coefficient of variation) for each of the human serum total CO concentration standards’ (12, 21, and 31 mmol of CO/L) measured in triplicate were 11.82 ± 0.65 mmol/L (5.5%), 21.11 ± 0.87 mmol/L (4.1%), and 30.75 ± 1.22 mmol/L (4.0%), respectively, for 20 analytic runs.

Once the calibration requirements were met, an evacuated tube was randomly selected with a random number generator and its top was removed. Then, 0.5 mL of plasma was gently aspirated with a plastic transfer pipette and transferred to the measuring cup of the total CO analyzer. Total CO concentration was measured in triplicate within 5 minutes after cap removal, and the mean total CO concentration was calculated. The mean of 3 sequentially determined values was deemed an appropriate value to report because total CO concentrations were equivalent among the first (29.9 ± 2.0 mmol/L), second (30.1 ± 2.0 mmol/L), and third (30.0 ± 2.0 mmol/L) sample measurements.

**Statistical analysis**—Data are reported as mean ± SD. Regression analysis was used to determine the linear association between the calculated volume of blood samples and measured weight of those samples and between mean total CO concentration and evacuated tube pressure. Repeated-measures ANOVA with a compound symmetry covariance structure was used to examine the fixed effects of tube type on plasma total CO concentration, with horse included as a random effect. Bonferroni adjusted posttest comparisons were conducted when a significant \( F \) test association with tube type was identified. Multivariate regression analysis was used to determine the linear association between plasma total CO concentration and the air volume–to–blood volume ratio, with dummy variables used to represent each horse. This ANCOVA approach accounts for between-subjects variability, thereby increasing the precision with which slope and intercept coefficients for the regression line can be estimated. Dummy variables (\( H_1 \) through \( H_7 \)) were defined as described by Noel et al. Values of \( P < 0.05 \) were considered significant for all analyses.

**Results**

**Air volume–to–blood volume ratio**—The calculated blood volume for the last 10 horses from which blood samples were collected was identical to the measured blood weight for the 80 evacuated tubes collected (\( R^2 = \)
0.97; intercept, 0.00 g; coefficient, 1.01; \( P = 0.96 \) and 0.65, compared with the intercept [0] and slope [1] values for the line of identity, respectively). This validated our use of the distance from the top of the blood to the bottom of the rubber stopper to calculate blood volume and air volume. The calculated volume of blood in each evacuated tube differed from the specified volume to a different degree for each tube (Table 1). The air volume–to–blood volume ratio was markedly higher (\( P < 0.001 \)) in 2-mL glass, 2-mL plastic, and 3-mL plastic tubes than the other 5 evacuated tube types (10-mL glass, 3-mL plastic plasma separator, 10-mL plastic, 6-mL plastic, and 4-mL plastic tubes), which had statistically equivalent ratios (Figure 1).

**Evacuated tube pressure**—Different mean partial vacuum pressures were measured in 6 types of evacuated tubes (Table 1). A negative linear relationship (\( R^2 = 0.62 \)) was detected between mean total CO\(_2\) concentration and evacuated tube pressure (cm H\(_2\)O). The regression equation for total CO\(_2\) concentration was \(-0.011 \times \) pressure + 28.5 (Figure 2). The air volume–to–blood volume ratio was lower in tubes with the greatest negative pressure.

**Total CO\(_2\) concentration**—A significant effect of evacuated tube size on the mean value for plasma total CO\(_2\) concentration was evident. Total CO\(_2\) concentration was significantly (\( P < 0.001 \)) lower in the 2-mL plastic and 2-mL glass tubes than in the other 6 types of evacuated tubes (Figure 3). Total CO\(_2\) concentration in the 3-mL plastic tubes was lower than that in the

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**Table 1**—Characteristics of various types of evacuated tubes used to collect blood samples from 20 horses.

<table>
<thead>
<tr>
<th>Tube type</th>
<th>Dimensions (mm)</th>
<th>Calculated blood volume (mL)</th>
<th>Interior tube pressure (cm H(_2)O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-mL glass</td>
<td>13 ( \times ) 75</td>
<td>1.9 ( \pm ) 0.5(^a)</td>
<td>(-80.8 \pm 1.4)^a</td>
</tr>
<tr>
<td>2-mL plastic</td>
<td>13 ( \times ) 75</td>
<td>2.2 ( \pm ) 0.5(^a)</td>
<td>(-72.2 \pm 2.7)^a</td>
</tr>
<tr>
<td>3-mL plastic</td>
<td>13 ( \times ) 75</td>
<td>2.4 ( \pm ) 0.2(^a)</td>
<td>(-109.9 \pm 5.8)^a</td>
</tr>
<tr>
<td>3-mL plastic</td>
<td>13 ( \times ) 75</td>
<td>3.4 ( \pm ) 0.2(^a)</td>
<td>(-113.1 \pm 0.7)^a</td>
</tr>
<tr>
<td>plasma separator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-mL plastic</td>
<td>13 ( \times ) 75</td>
<td>4.8 ( \pm ) 0.3(^a)</td>
<td>(-144.2 \pm 1.1)^a</td>
</tr>
<tr>
<td>8-mL plastic</td>
<td>13 ( \times ) 100</td>
<td>6.5 ( \pm ) 0.5(^a)</td>
<td>(-202.5 \pm 2.9)^a</td>
</tr>
<tr>
<td>10-mL glass</td>
<td>16 ( \times ) 100</td>
<td>9.3 ( \pm ) 0.4(^d)</td>
<td>ND</td>
</tr>
<tr>
<td>10-mL plastic</td>
<td>16 ( \times ) 100</td>
<td>10.2 ( \pm ) 0.3(^d)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values represent mean \( \pm \) SD for 5 to 10 tubes from the same lot number.

\( ^a \) Within a column, values with different superscript letters are significantly (\( P < 0.05 \)) different.

ND = Not determined.

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**Figure 1**—Histogram of mean \( \pm \) SD air volume–to–blood volume ratio versus tube size (A) and scatterplot (B) of plasma total CO\(_2\) concentration as a function of air volume–to–blood volume ratio for 20 healthy horses from each of which jugular venous blood was collected into 8 evacuated tubes of various sizes. B—The thick curvilinear line represents the linear regression line for plasma total CO\(_2\) concentration as a function of air volume–to–blood volume ratio (\( y = -0.57x + 30.54 \)). Thin horizontal lines represent the 95% confidence interval (30.40 to 30.67 mmol/L) for the intercept value. The dashed vertical line represents the value for air volume–to–blood volume ratio 0.21 when the fitted regression line first appears lower than the 95% confidence interval for the intercept value. P = Plastic evacuated tube. G = Glass evacuated tube. PP = Plastic plasma separator tube.

**Figure 2**—Mean \( \pm \) SD air volume–to–blood volume (top panel) and plasma total CO\(_2\) concentrations (bottom panel) in the horses in Figure 1 as a function of mean evacuated tube pressure. The solid line represents the linear regression line (\( y = -0.011x + 28.5 \)), and the dashed lines represent the 95% confidence interval.

**Figure 3**—Mean \( \pm \) SD plasma total CO\(_2\) concentrations of 20 healthy horses from each of which jugular venous blood was collected into 8 evacuated tubes of various sizes. Notice that the \( x \) axis starts at 28 mmol/L. Inter = Intercept value for the related linear regression equation. \( ^a \) Mean values with different letters are significantly (\( P < 0.05 \)) different. See Figure 1 for remainder of key.
3-mL plastic plasma separator, 6-mL plastic, 4-mL plastic, 10-mL glass, and 10-mL plastic tubes. Concentrations in the 6-mL plastic, 4-mL plastic, 10-mL glass, and 10-mL plastic tubes were statistically similar. Calculated decreases in total CO₂ concentration from the theoretically true (intercept) value were 5.8%, 5.5%, 2.8%, 1.3%, 1.2%, 0.7%, 0.5%, and 0.1% for the 2-mL plastic, 2-mL glass, 3-mL plastic, 3-mL plastic plasma separator, 6-mL plastic, 4-mL plastic, 10-mL glass, and 10-mL plastic tubes, respectively. A strong linear relationship (R² = 0.92) was evident between total CO₂ concentration and air volume–to–blood volume ratio (Figure 1). The least squares mean value for the intercept (equivalent to zero air volume or blood collected anaerobically into a syringe) of that regression equation was 30.7 mmol/L.

Discussion

To the authors’ knowledge, the present study is the first in which the effect of evacuated tube size on the measured plasma total CO₂ concentration in horses was examined. The major finding was that the size of the evacuated tube used for blood collection influenced the measured total CO₂ concentration, even when care was taken to ensure complete filling of each tube. An additional finding was that there was a significant effect of the type of evacuated tube (plastic vs glass) on sample volume in the 2- and 10-mL tubes, with plastic tubes having a larger fill volume than glass tubes, as represented by increased sample volume (Table 1). However, this difference in draw volume did not translate to a significant difference in the measured total CO₂ concentration for equivalent-sized tubes.

Heparinized plastic evacuated tubes have been used extensively to collect jugular venous blood samples for determination of total CO₂ concentration for detection of alkalinizing agents in horses before racing.7,8,11–13 Heparinized evacuated tubes have also been used to collect jugular venous blood samples for blood gas and acid-base analysis,14–16 despite the fact that the 2009 Clinical and Laboratory Standards Institute document on approved guidelines for blood gas and pH analysis does not recommend the use of evacuated tubes for sample collection,17 and Mueller and Lang18 state that “the use of Vacutainers for determining P(O₂) and percentage hemoglobin saturation is condemned.”

Collecting blood samples into plastic evacuated tubes has some disadvantages for measurement of total CO₂ concentration because of the semipermeable properties of plastic and the inability to maintain an anaerobic sample.17 Carbon dioxide is lost from the blood sample into the gas phase above the sample within the evacuated tube,18 or into the atmosphere after opening the evacuated tube. Fluids in an evacuated tube are subject to the fundamental gas laws such that the behavior of the liquid and gas phases will be influenced by partial pressure gradients and solubilities; therefore, a liquid and a gas in a closed container will come to equilibrium.18 This loss of CO₂ from the blood sample leads to a subsequent decrease in blood P(CO₂) increase in blood pH, and the potential for alteration of the calculated values for HCO₃⁻ concentration, total CO₂ concentration, and base excess in extracellular fluid, which are 3 widely used indices of the strong ion (metabolic) component of an acid-base disturbance.19,20 However, Noel et al10 recently reported that the HCO₃⁻ concentration, total CO₂ concentration, and base excess in extracellular fluid calculated from the results of blood gas and acid-base analysis of equine blood were not altered by collection in plastic evacuated tubes, compared with values obtained when a similar blood sample was anaerobically collected in a glass syringe.

In the present study involving blood samples from 20 horses, the mean intercept value for plasma total CO₂ concentration was 30.7 ± 1.9 mmol/L. The mean total CO₂ concentration was similar to those previously reported for similar collection and analytic methods: a total CO₂ concentration of 30.8 ± 1.4 mmol/L11 and 31.5 mmol/L11 for racehorses in Australia, 30.2 ± 1.2 mmol/L for Australian horses,16 and 31.1 mmol/L for male Thoroughbred racehorses and 30.7 mmol/L for female Thoroughbred racehorses.13

The mean pressure in the evacuated tubes evaluated in the present study varied with the size of the tube, with values ranging from −72 (2-mL plastic tubes) to −203 cm H₂O (6-mL plastic tubes). We did not investigate whether there were interlot differences in evacuated tube pressure. Our pressure measurements were similar to previously measured mean values of −72 cm H₂O for 5-mL serum tubes,21 −102 cm H₂O for 10-mL serum tubes,21 approximately −102 cm H₂O for evacuated tubes,22 and −320 cm H₂O for evacuated tubes.23 The quantity of blood drawn into an evacuated tube is primarily dependent on the difference between the evacuated tube pressure and venous pressure but also varies with altitude, ambient temperature, barometric pressure, tube age, and filling technique.24 It should be considered that blood is usually obtained from humans after application of a tourniquet that increases IV pressure to 40 mm Hg,22 whereas blood samples are usually obtained from the jugular vein of horses without venous occlusion during collection, and the mean jugular venous pressure is reportedly 7.4 mm Hg.23 This means that the pressure gradient for filling evacuated tubes designed for use in humans is much lower in horses and as a consequence, it is possible that the common practice of not using a tourniquet in horses results in lower draw volumes in evacuated tubes.

The only plasma separator tube we examined (3-mL plastic plasma separator tube) had significantly better fill, compared with the 3-mL plastic tube, which resulted in a significant, albeit small, increase in total CO₂ concentration. On the basis of the results of our study, whenever an accurate estimate of total CO₂ concentration is required, a blood sample should be collected into the evacuated tube with the smallest air volume–to–sample volume ratio, which translates to the largest evacuated tube with the most negative pressure. The choice of evacuated tube size is particularly important when the measured plasma total CO₂ concentration is to be compared with values calculated from the results of blood gas and acid-base analysis of an anaerobically collected sample. When small (2- or 3-mL) evacuated tubes must be used, plastic separator tubes might result in more accurate results for measured total CO₂ concentration.
concentration. However, other comparisons of plastic or glass separator tubes with plastic or glass evacuated tubes of the same size would be needed to draw more definitive conclusions about their impact on total CO₂ concentration.

a. Random number generation, Data Analysis Add-In, Microsoft Office Excel 2003, Redmond, Wash.
b. Vacutainer, No. 367214, Becton Dickinson, Franklin Lakes, NJ.
c. Vacutainer, No. 367671 (13 × 75 mm), Becton Dickinson, Franklin Lakes, NJ.
d. Vacutainer, No. 366664 (13 × 75 mm), Becton Dickinson, Franklin Lakes, NJ.
e. Vacutainer, No. 366667 (13 × 75 mm), Becton Dickinson, Franklin Lakes, NJ.
f. Vacutainer, No. 367960 (13 × 75 mm), Becton Dickinson, Franklin Lakes, NJ.
g. Vacutainer, No. 367884 (13 × 75 mm), Becton Dickinson, Franklin Lakes, NJ.
h. Vacutainer, No. 367886 (13 × 100 mm), Becton Dickinson, Franklin Lakes, NJ.
i. Vacutainer, No. 366480 (16 × 100 mm), Becton Dickinson, Franklin Lakes, NJ.
j. Vacutainer, No. 367880 (16 × 100 mm), Becton Dickinson, Franklin Lakes, NJ.
k. 16-gauge, 1-inch needle, Becton Dickinson, Franklin Lakes, NJ.
l. DP 45-34 transducer, Validyne Engineering Corp, Northridge, Calif.
m. Pulmonary Mechanics Analyzer, XA version, Buxco Electronics Inc, Sharon, Conn.
n. Beckman Synchron EL-ISE, Beckman Coulter Inc, Brea, Calif.
o. Verichem Laboratories Inc, Providence, RI.
q. PROC REG, SAS, version 9.2, SAS Institute Inc, Cary, NC
r. Beckman Coulter Inc, Brea, Calif.
s. PROC MIXED, SAS, version 9.2, SAS Institute Inc, Cary, NC.

References