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Venous blood gas parameters, electrolytes, glucose and lactate concentration in sick neonatal foals: direct venipuncture versus push-pull technique

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Venous blood gas parameters, electrolytes, glucose and lactate concentration in sick neonatal foals: direct venipuncture versus push-pull technique

Chiara Del Prete^{1*}, Aliai Lanci², Natascia Cocchia¹, Francesca Freccero², Chiara Di Maio², Carolina Castagnetti^{2,3}, Jole Mariella², Fabiana Micieli¹

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Venous blood gas parameters, electrolytes, glucose and lactate concentration in sick neonatal foals: direct venipuncture versus push-pull technique

Running head: Blood gas values: venipuncture vs push-pull technique

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Declarations

a. Authorship

The authors listed as part of the manuscript have substantially contributed to the study design and execution, data analysis and interpretation, preparation and approval of the manuscript and gave final approval of the version to be published.

b. Source of Funding

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c. Competing interests

The authors have declared no competing interests

d. Ethical Animal Research

No specific ethical approval was required since this observational study did not modify current diagnostic or therapeutic strategies.

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e. Owner informed consent

Signed client consent was obtained for each horse.

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Keywords: foal, intravenous catheter collection, venous blood analysis

Summary

Background: Blood collection by indwelling intravenous catheter (IVC) was proposed to reduce repeated venipuncture, which could cause thrombophlebitis risk, anxiety and pain in patients.

Objectives: To compare blood gas parameters, electrolytes, glucose, lactate and hematocrit concentration obtained from venous blood samples collected via a jugular IVC by push-pull (PP) technique to those obtained by venipuncture in hospitalized foals, at the time of catheter placement (T0) and 24 hours after the beginning of intravenous therapy (T24).

Study design: Prospective observational study.

Methods: Paired blood samples were drawn from hospitalized foals at T0 and T24. For each foal, one venous blood sample was collected via IVC by the following PP technique: 2.4 mL of blood was aspirated and immediately reinfused through the catheter 3 times consecutively, then 1 mL of blood was collected using a 1 mL heparinized syringe. Thereafter, another sample was collected by direct venipuncture of the contralateral jugular vein, with an identical 1 mL heparinized syringe, equipped with a 1-inch, 20-G needle. All blood samples were analyzed with an automated blood gas analyzer within 10 minutes of collection. The agreement between the two techniques was assessed by Bland – Altman analysis and intraclass correlation coefficient (ICC).

Results: The level of agreement of blood gas values obtained by the two different techniques was high with very small bias and ICC clinically acceptable (>0.907 at T0; >0.794 at T24) for all variables, except for Hct (bias -3.52 at T0; -2.44 at T24) and PvO_2 at T0 and T24 (ICC 0.669 and 0.733 , respectively).

Main limitations: Sub-clinical catheter-related complications were not investigated by ultrasound or bacterial culture of the catheter; short duration of the study. **Conclusions:** PP technique appears to be

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acceptable for collection of blood samples for venous blood gas parameters, as well as electrolytes, glucose and lactate in sick neonatal foals.

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Introduction

In horses, repeated venipuncture of the jugular vein is likely to be associated with vessel trauma and risk of developing thrombophlebitis [1,2]. This method has the potential to reduce not only repeated venous trauma but also stress, anxiety and pain experienced by patients [3,4]. Hospitalized, critically-ill neonatal foals usually require multiple blood sampling for clinical evaluation and monitoring. Blood collection is often performed by direct venipuncture despite the presence of an indwelling intravenous catheter (IVC).

In veterinary medicine, the effect of blood collection by IVC on blood parameters is limited to a few studies [3, 5-10]. In horses, May et al. [7] demonstrated that hematologic and biochemical values obtained from blood samples collected via IVC are comparable to those obtained from blood collected by direct venipuncture. Moreover, clinical equivalence of coagulation parameters has been reported between the two techniques in dogs and horses [3,5,8,10]

In foals, venous blood gas parameters are successfully used for monitoring neonatal adaptation and patients under intensive care [11,12]. The venous concentrations of acid-base parameters and electrolytes, glucose and lactate are also routinely evaluated and are reliable as long as the blood is obtained from a vein without stasis [13-15].

Recently, Barr et al. [9] showed that in dogs, the push-pull (PP) technique from an IVC did not affect the blood gas results compared to those obtained by direct venipuncture. To the authors' knowledge, there are no studies comparing blood gas values obtained from blood samples collected by direct venipuncture or by the PP technique in horses.

The main aim of this study was to compare venous blood gas parameters, electrolytes, glucose, lactate and hematocrit concentrations obtained from blood samples collected by PP technique through a jugular catheter with those sampled by direct jugular venipuncture in hospitalized foals. The second aim was to evaluate if blood values from the two collecting techniques would differ after receiving

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intravenous treatments via IVC for 24 hours. We hypothesized that the evaluated parameters obtained by samples collected with the two different techniques would not significantly differ. Further, PP sampling technique after 24 hours of catheter use for IV therapy would not have significant effects on the evaluated values.

Material and methods

This study was a prospective observational study. It included sick foals within 30 days of age, born at or admitted to the Equine Perinatology and Reproduction Unit of the Department of Veterinary Medical Sciences (University of Bologna), which received a jugular catheter placement for IV therapy and blood gas analysis monitoring as part of their diagnostic or therapeutic plan. The specific intravenous treatment protocol was dependent on the foal's clinical status. For all subjects, signalment (breed, age, sex, and bodyweight), diagnosis and treatments were recorded. Both jugular veins were inspected and manually checked twice a day during the hospitalization time to determine the presence of alterations.

Blood sample collection

At the admission of the foals, a long-term 20 cm, 16-G, IVC [LOGICATH™]^a was aseptically placed into one randomly chosen jugular vein. Immediately after the catheterization (T0), a blood sample was collected through the IVC by PP technique as described by Barr et al. [9]. Prior to insertion, the dead space of the catheter and connected extension set were calculated by use of the saline solution displacement technique, being 0.6 mL and 0.2 mL respectively. For the PP technique, a 10 mL syringe was used to aspirate a volume of blood equal to 300% of the dead space volume of catheter and extension set (2.4 mL). Such blood volume was immediately reinfused into the jugular vein without disconnecting the syringe or extension set from the catheter. This procedure was repeated 3 times before connecting a one-mL heparinized syringe with 50 IU of Heparin Calcium Balanced/mL of

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blood [Monovette®]^b to collect venous blood. The IVC was flushed with 1 mL of 0.5% heparinized saline solution before and after each sampling procedure. Thereafter, a venous sample collection was performed by direct venipuncture of the contralateral jugular vein using the same type of heparinized syringe [Monovette®]^b equipped with a 1-inch 20 Gauge needle. After 24 hours from catheter placement (T24), the paired blood samples were collected employing that same protocol. For those foals did not received continuous IV fluid therapy, medication infusion and/or catheter flushing with 1 mL of 0.5% heparinized saline solution were repeated at least every 6 hours. For those foals undergoing continuous fluid therapy, the IV infusion was stopped 5 minutes before the paired blood sampling [7]. All blood samples were analyzed by an automated blood gas analyzer machine [Roche Opti CCA]^c within 10 minutes from sampling. Haematocrit (Hct %), pH, PvO₂ (mmHg), PvCO₂ (mmHg), K⁺ (mmol/L), Na⁺ (mmol/L), Ca²⁺ (mmol/L), Cl⁻(mmol/L), Anion gap (mmol/L), Osm (mOsm/kg), Glucose (mmol/L), Lactate (mmol/L), HCO₃ (mmol/L), Actual base excess (ABE; mmol/L), Standard base excess (SBE; mmol/L) were recorded on a datasheet for each sample. Hct is a calculated value derived by measured haemoglobin.

Statistical analysis

Venous blood gas parameters, electrolytes, glucose, lactate and hematocrit concentrations were tested for normal distribution using histograms and Shapiro-Wilk test and reported as mean ± SD or median (1st quartile, 3rd quartile) if normally or not normally distributed, respectively. The level of agreement between the two techniques was evaluated using the Bland-Altman plot [16]. The bias (mean difference) and 95% of interval confidence between the two techniques were calculated according to the method of Bland and Altman. The intraclass correlation coefficient (ICC) was used to assess the agreement between the two techniques for all evaluated blood gas parameters. ICC values below 0.50

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were considered to show poor agreement, values ranging between 0.50 and 0.75, 0.75 and 0.90 and above 0.90 were considered to reflect respectively moderate, good and excellent agreement [17].

Statistical analyses were performed using both SPSS 25.0^d and Analyze-It for MS Excel^e.

Results

Seventeen foals, ten colts and seven fillies, of different breeds (8 Standardbreds, 3 KWPN, 2 Arabians, 2 Thoroughbreds, 1 Holstein and 1 Bardigiano), ranging in bodyweight from 35 to 93 kg (54.3 ± 14.3), were included in this study. Their age at T0 ranged from 8 hours to 21 days (median; 32.5 hours). Although many foals suffered from multiple pathologies, the main diagnoses of patients were: perinatal asphyxia syndrome (6/17), omphalitis (5/17), meconium retention (5/17), and sepsis (5/17); other diagnoses included: neonatal isoerythrolysis (1/17), enteritis (1/17), guttural pouch tympany (1/17) and haemorrhagic shock (1/17). Treatment plan included IV broad-spectrum antimicrobials (ampicillin and amikacin) (16/17), vitamins (8/17), flunixin meglumine (7/17), equine plasma (7/17), Lactated Ringer's solution^f (6/17), and 5% or 10% dextrose solution^g (6/17) administered on a case by case basis.

At 24 hours, two foals were excluded because of spontaneous death. Meanwhile in three other foals it was not possible to withdraw the blood through the catheter, with unimpeded capacity for infusion of fluids and without macroscopical vein problems. There were no catheter-related infections of the jugular vein or complications due to the direct venipuncture identified during hospitalization in the foals involved in this study. None of the collected samples was coagulated or haemolysed.

Mean \pm standard deviation (SD) or median (1st quartile, 3rd quartile) values of venous blood gas parameters collected by direct venipuncture and by push-pull technique are reported in Table 1.

Bland Altman analysis revealed no significant fixed bias between paired venipuncture and PP-techniques samples. Mean differences (biases) calculated for all blood parameters were fairly small,

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except for Hct and PvO₂ values at both T0 and T24 (Supplementary items 1 and 2). All bias and 95% limits of agreement are reported in Table 1 for T0 and in Table 2 for T24. Bland-Altman plots analysis showed a close agreement between the results obtained by the two sampling methods for almost all parameters. The limits of agreement were wide only for Hct and PvO₂ values in both T0 and T24 samples, as shown in Figure 1 and 2, respectively.

In the majority of the cases (80% at T0 and 73% at T24) ICC values were above 0.90 agreement, as reported in Table 1 and 2, respectively, demonstrating an excellent agreement between the two methods. As shown in Table 1 and 2, the correlation coefficient between the two methods was moderate only for PvO₂ at both T0 and T24, while it was good for Hct at T0 and for pH, anion gap and glucose at T24.

Discussion

Blood sampling from IVCs is not uniformly used in clinical practice and there are no data on the reliability of this technique in foals. The results of our study confirm the opportunity to adopt the PP technique to collect blood samples to perform venous blood gas analysis in foals.

Venous blood sampling by PP technique can be quickly and easily performed, reducing the anxiety and pain experienced by the foal and the use of physical restraint, the related risks for the operator and the number of personnel involved in the procedure. Moreover, this technique may drastically reduce vessel trauma and risk of developing thrombophlebitis due to repeated venipuncture, especially in foals with limited venous accesses and in critically ill foals that require serial monitoring. Some authors reported that the risk of catheter and injection site related infections or thrombosis is higher in foals hospitalized for sepsis [18,19]. In this study, despite the presence of 30% of septic foals, it has been shown that the presence of a jugular catheter provided a ready and easy venous access without any clinically evident complication in none of the animals. However, the occurrence of early sub-clinical catheter-related complications (i.e microthrombi, contaminations, fibrin sheaths)

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cannot be excluded, as these were not assessed by ultrasound [20] or bacterial culture of the catheter. In adult horses, ultrasonographic monitoring of jugular veins subjected to repeated venipuncture or during jugular catheterization is recommended to detect early abnormalities and to reduce the incidence of thrombophlebitis [20,21].

Three different techniques for drawing a blood sample from a catheter are reported in the medical literature: the discard, the reinfusion, and the “mixing” or PP methods [22]. The discard technique is the most commonly used in human hospital settings, which involves aspirating and discarding 3 to 10 mL of blood to clear the catheter; then a brand-new syringe is used to draw the final sample for analysis. The reinfusion technique requires that the blood is returned to the patient after performing the blood collection for the analysis. The disadvantages of the “discard” and reinfusion methods are the significant blood loss and a higher risk of contamination, respectively. In the authors’ opinion, the PP technique used in the present study reduces both of these risks. However, the vortex created during blood aspiration may increase the risk of haemolysis [22]. There is evidence that haemolysis is inversely correlated with catheter’s diameter, being very low when this is ≤ 16 G in both human and horses [7,23]. No evidence of haemolysis of the samples collected by PP technique were found in human studies [22,24]. In our study, haemolysis was found in none of the samples at any time.

Our results suggest that IVC PP sampling technique is an acceptable method of collecting blood for the venous blood gas analysis in neonatal foals with an indwelling IV catheter. Although there are slight differences between blood gas parameters, as well as electrolytes, glucose, lactate and hct concentrations, collected by venipuncture and by PP technique, these differences were considered clinically acceptable for serial monitoring, except for Hct and PvO₂. This finding is in agreement with a similar study that proposed PP technique in dogs under general anesthesia [9].

In the present study, the only two values with a clinically unacceptable difference were PvO₂ and Hct, both at the time of catheter placement and after 24 hours. A discordant trend between the two

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collection's techniques at two time points was observed for those two values, for which it is difficult to find an explanation. Further investigation in a larger sample might provide new insights. A similar study conducted by Barr et al. [9] in dogs found no differences in Hct between samples collected by venipuncture and PP technique. In dogs, blood collection was performed from cephalic vein and the two samplings were performed in the same blood flow direction. Conversely, in the present study, blood was aspirated in different directions respect to the blood flow by the two methods. Whether this factor, together with the different caliber of the needle and catheter, might have an effect on the findings is not clear from this study. However, evaluation of PvO₂ and Hct from venous blood is rarely utilized in daily practice [13]. In clinical practice, venous O₂ tension is not helpful in the assessment of pulmonary function and the arterial blood gas is considered the gold standard methods for assessment of pulmonary function in foals [25]. Moreover, the use of Hct from blood gas analyzer was recently discouraged [26].

In the present study, blood gas parameters, as well as electrolytes, glucose and lactate concentrations, obtained through the PP respect to venipuncture were still comparable after 24 hours of IV treatments, except again for PvO₂ and Hct.

Similarly, in previous study, no differences were found for haematological and biochemical values between the same methods of blood sampling in horses receiving fluid therapy or low volume intravenous medications for up to two weeks [7].. To authors' knowledge, no complications in blood collection by IVC have been reported in veterinary literature. In this study, in three cases despite the drugs infusion was unimpeded through the catheter, blood collection by PP technique was unworkable. We hypothesized that the tip of the catheter was touching the wall of the vein or that the negative pressure exerted by the syringe aspiration caused the occlusion of the catheter lumen during such maneuver. The unimpeded capacity for infusion of fluids accompanied by the impossibility of blood collection from the catheter is already reported in human medicine as “persistent withdrawal

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occlusion” [27]; it is frequently explained by the presence of fibrin sheath around the catheter tips acting as a one way valve [27-29].

The present study was observational and the intravenous treatments were not standardized, as they were dependent upon the individual’s disease. Uniformity of the IV treatment plan between foals was beyond the aim of this study and unfeasible in the clinical setting of the study. This high variability between subjects was minimized by the use of paired samples and it could reproduce the real clinical situation. An important limitation of this study was the short duration of time (24 hours). As previously discussed, longer-term effects of the PP technique on catheter-related complications warrants further investigations; and moreover, the sample size might be increased to clarify the inconsistency in PvO₂ and Hct results.

In conclusion, blood gas variables (pH, PvCO₂, HCO₃, ABE, SBE), electrolytes, glucose and lactate concentrations from samples obtained by PP technique via IVC were comparable to those obtained by direct venipuncture, indicating that this is an acceptable method for routine blood gas analysis and monitoring in critically-ill neonatal foals during the first 24 hours of catheterization.

Manufacturers’ addresses

^a Smiths Medical Inc, Ashford, UK

^b Sarstedt, Nümbrecht, Germany

^c Roche Diagnostic Corporation, Indianapolis, IN, USA

^d SPSS Inc. Chicago, IL, USA

^e Analyze-It Software Ltd, Leeds, UK

^f S.A.L.F. S.p.A, Bergamo, Italy

^g B Braun S.p.A., Milano, Italy

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Table 1. Venous blood gas parameters, electrolytes, glucose, lactate and hematocrit concentration obtained at the time of catheter placement (T0) by direct venipuncture and push-pull technique in 17 foals are expressed as mean \pm SD or median (1st quartile, 3rd quartile) if normally or not normally distributed, respectively. Bland Altman analysis showing bias (mean difference) and 95% limits of agreement and Intra class correlation (ICC) values between direct venipuncture and push-pull technique are reported. ICC values: >0.90 excellent, 0.75–0.90 good, 0.50–0.75 moderate, and <0.5 poor agreement.

Blood parameter	Blood collection technique		Bland Altman Analysis		ICC values
	Direct venipuncture	Push-pull technique	Bias (mean difference)	95% limits of agreement	
Hematocrit (%)	38.6 \pm 12	35 \pm 12.6	-3.52	-8.8 to 1.77	0.779
pH	7.33 \pm 0.07	7.35 \pm 0.06	0.02	-0.001 to 0.034	0.918
PvO ₂ (mm Hg)	54.2 (42.9, 60.6)	39.8 (35.7, 57)	-5.84	-12.45 to 0.76	0.669
PvCO ₂ (mm Hg)	51.6 \pm 6.4	52.9 \pm 5.2	-1.31	-2.98 to 0.37	0.907
K ⁺ (mmol/L)	3.7 (3.5, 4.3)	3.7 \pm 0.7	0.28	0.14 to 0.42	0.939
Na ⁺ (mmol/L)	133 (132, 137)	134 (131.7, 137.7)	0.1	-0.3 to 0.4	0.997
Ca ²⁺ (mmol/L)	1.5 \pm 0.1	1.5 (1.4, 1.5)	0.01	0.004 to 0.03	0.979
Cl ⁻ (mmol/L)	100 (97.7, 104.3)	100 (98.7, 104.3)	-0.18	-0.59 to 0.24	0.995
Anion gap (mmol/L)	11.5 \pm 5.1	11.5 \pm 4.7	0.02	-0.6 to 0.64	0.986
Osmolarity (mOsm/Kg)	274.5 (270.6, 282.1)	277.5 (270.1, 281.6)	0.08	-0.71 to 0.87	0.997
Glucose (mmol/L)	7.8 \pm 1.8	7.7 \pm 1.7	0.06	-0.17 to 0.30	0.984
Lactate (mmol/L)	2.4 (1.5, 5.8)	2.4 (1.5, 5.6)	-0.05	-0.18 to 0.09	0.998
HCO ₃ (mmol/L)	25.6 \pm 3.8	25 (23.4, 27.8)	0.72	0.49 to 0.94	0.988

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Actual base excess (mmol/L)	1.9 (-0.3, 4.3)	0.8 (-1.1, 4)	0.9	0.6 to 1.2	0.987
Standard base excess (mmol/L)	2.5 (0.63, 4.9)	2.5 (-0.3, 4.3)	0.71	0.46 to 0.96	0.992

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Table 2. Venous blood gas parameters, electrolytes, glucose, lactate and hematocrit concentration obtained 24 hours after catheter placement (T24) by direct venipuncture and push-pull technique for 17 foals are expressed as mean \pm SD or median (1st quartile, 3rd quartile) if normally or not normally distributed, respectively. Bland Altman analysis showing bias (mean difference) and 95% limits of agreement and Intra class correlation (ICC) values between direct venipuncture and push-pull technique are reported. ICC values: >0.90 excellent, 0.75–0.90 good, 0.50–0.75 moderate and <0.5 poor agreement.

Blood parameters	Blood collection technique		Bland Altman Analysis		ICC Values
	Direct venipuncture	Push-pull technique	Bias (mean difference)	95% limits of agreement	
Hematocrit (%)	34.3 \pm 12.7	36.7 \pm 9.1	-2.44	-6.59 to 1.72	0.910
pH	7.36 \pm 0.04	7.35 \pm 0.04	-0.013	-0.002 to 0.028	0.885
PvO ₂ (mm Hg)	47.1 \pm 12.3	51.2 (45.2, 56.2)	-7.12	-14.48 to 0.25	0.733
PvCO ₂ (mm Hg)	51.5 \pm 8.2	52.4 \pm 9.1	-0.94	-3.42 to 1.54	0.954
K ⁺ (mmol/L)	3.9 \pm 0.4	4.1 (3.8, 4.2)	-0.02	-0.15 to 0.11	0.942
Na ⁺ (mmol/L)	134.7 \pm 1.9	134.2 \pm 1.8	0.45	-0.24 to 1.15	0.910
Ca ²⁺ (mmol/L)	1.5 \pm 0.07	1.5 \pm 0.07	0.014	-0.005 to 0.032	0.961
Cl ⁻ (mmol/L)	100 (98.2, 103.8)	101 (98, 103.7)	0.00	-0.52 to 0.52	0.994
Anion gap (mmol/L)	9.5 \pm 1.9	9.2 \pm 1.6	0.28	-0.46 to 1.02	0.895
Osmolarity (mOsm/Kg)	278.3 \pm 3.9	278.1 \pm 4.1	0.23	-1.19 to 1.64	0.930
Glucose (mmol/L)	9 \pm 1.8	9.5 \pm 2.2	-0.48	-1.61 to 0.65	0.794
Lactate (mmol/L)	1.85 \pm 0.6	1.85 \pm 0.6	0.01	-0.23 to 0.25	0.913
HCO ₃ (mmol/L)	26.5 \pm 2.8	25.9 \pm 2.5	0.57	-0.07 to 1.22	0.957

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Actual base excess (mmol/L)	2.4 ± 2.8	2 ± 2.9	0.45	0.06 to 0.85	0.984
Standard base excess (mmol/L)	2.9 ± 2.9	2.5 ± 3.1	0.4	0.01 to 0.79	0.987

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Figure legends

Figure 1. Bland Altman plots for comparison of Hct (A) and PvO₂ (B) of samples collected by direct venipuncture (V) and those collected by the push-pull (PP) technique from 17 foals at time of catheter placement (T0); the solid line represents the bias (mean difference between two techniques) and the two dotted lines the 95% limits of agreement (mean ± 1.96 SD).

Figure 2. Bland Altman plots for comparison of Hct (A) and PvO₂ (B) of samples collected by direct venipuncture (V) and those collected by the push-pull (PP) technique from 17 foals after 24 hours of catheter placement (T24); the solid line represents the bias (mean difference between two techniques) and the two dotted lines the 95% limits of agreement (mean ± 1.96 SD).

Supplementary items legends

Supplementary item 1. Bland Altman plots for comparison of all blood gas parameters of samples collected by direct venipuncture (V) and those collected by the push-pull (PP) technique from 17 foals at time of cathether placement (T0): pH (A), PvCO₂ (B), K⁺ (C), Na⁺ (D), Ca²⁺ (E), Cl⁻ (F), Anion gap (G), Osm (H), Glucose (I), Lactate (J), HCO₃ (K), ABE (L), SBE (M); the solid line represents the bias (mean difference between two techniques) and the two dotted lines the 95% limits of agreement (mean ± 1.96 SD).

Supplementary item 2. Bland Altman plots for comparison of pH (A), PvCO₂ (B), K⁺ (C), Na⁺ (D), Ca²⁺ (E), Cl⁻ (F), Anion gap (G), Osm (H), Glucose (I), Lactate (J), HCO₃ (K), ABE (L), SBE (M) of samples collected by direct venipuncture (V) and those collected by the push-pull (PP) technique from 17 foals after 24 hours of cathether placement (T24); the solid line represents the bias (mean difference between two techniques) and the two dotted lines the 95% limits of agreement (mean ± 1.96 SD).

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