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Comparison of the blood gas analyser Edan i15 Vet with the blood gas analyser Gem Premier 3000 for the analysis of blood gases, electrolytes, hemoglobin and hematocrit in lactating Holsteins

MERT METIN[1], KEMAL AKSOY[2], AYTAÇ PEKMEZCI[3], ABDÜLKERIM DENIZ[4]

[1]Department of Biochemistry, [2]Department of Internal Medicine, Faculty of Veterinary Medicine Milas, University of Muğla Sıtkı Koçman, 48200 Milas, Muğla, Turkey
[3]Department of Statistics, Faculty of Science, University of Muğla Sıtkı Koçman, 4800 Kötekli, Muğla, Turkey
[4]Free Researcher for clinical biochemistry, Nisbetiye Mah., 34340 Beşiktaş, İstanbul, Turkey

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Metin M., Aksoy K., Pekmezci A., Deniz A.

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Summary

A point-of-care blood gas analyser, Edan i15 Vet (EDAN), was compared with a benchtop blood gas analyser, GEM Premier 3000 (GEM). GEM and EDAN were used to analyse whole blood from 123 lactating Holsteins within one month of calving for blood gases, electrolytes, hematocrit and hemoglobin. EDAN and GEM showed significant linear correlations for blood gases, electrolytes, Hct and Hb. The 95% confidence intervals (CI) of intercept in Passing-Bablok regressions included zero in pCO_2, bicarbonate, TCO_2, BE ecf, BE B, hematocrit, and pH, but not for Na^+, K^+, pO_2 and sO_2. The CI of the slope included 1.0 for Na^+, K^+, pCO_2, bicarbonate, TCO_2, BE ecf, BE B, hematocrit, and pH, but not for pO_2, sO_2, and hemoglobin. The Bland-Altman plots between EDAN and GEM showed a bias of 1.4% for Na^+, 2.4% for K^+, –1.6% for pCO_2, 3.0% for pO_2, –5.3% for bicarbonate, 2.8% for SO_2, –7.3% for TCO_2, 10.4% for Hct, 21.2% for Hb, –25.1% for BE B and –38.5% for BE ecf. The biases in the analysis of certain estimated parameters were much higher (> 5%) than for measured parameters except for Hct. Parity did not correlate with blood gas parameters but blood pH correlated negatively with K^+, pCO_2 and positively with pO_2, TCO_2, sO_2, bicarbonate, BE ecf and BE B. The postpartum time correlated positively with pCO_2, TCO_2, BE and bicarbonate and negatively with Hct and Hb values. Reference values (2.5-97.5% quartiles) were determined for each parameter. Conclusively, EDAN can be used interchangeably with GEM for the analysis of blood pH, K^+, pCO_2 and HCO_3^- as they show acceptable moderate agreement. However, they did not agree for other parameters such as TCO_2, BE ecf, BE B, Hb, Hct, sO_2, Na^+, and pO_2, therefore, reference values for each parameter were set for GEM and EDAN.

Keywords: blood gases, edan, gem, Holstein

Point-of-care (POC) blood gas analysers are used in humans to analyse important biochemical parameters in the organism, especially for rapid tests in the emergency room (4, 24, 26, 29). In dairy cows or calves, these devices can also provide rapid results so that appropriate treatment can be initiated quickly in the context of acute care. In dairy cows in particular, POC devices can be useful for rapid determination of blood gases such as pO_2, pCO_2, bicarbonate, base deficit and electrolytes to monitor physiological health status in acidosis and alkalosis, and bicarbonate ion loss in calves with diarrhoea (2, 8, 9, 25). The acid-base status of dairy cows is maintained within a narrow range and is associated with important biological functions of dairy cows (1). In particular, in recent years, the difference between cations and anions in the diet has been used for metabolic acidification in the finishing phase to stimulate the mobilisation of calcium from the bones to prevent milk fever (19). However, this acidification can also have fatal consequences, especially for the embryo (21). The maintenance of a normal pH in the blood is ensured by the buffer system; the HCO_3^-/H_2CO_3 system has an important role in this buffering process, which the organism can control by changing

the pCO_2 level through respiration and by controlling the bicarbonate ions in the blood (1). Therefore, monitoring acid-base status in cattle is a valuable diagnostic tool in dairy farming. Inappropriate nutrition is one of the main causes of metabolic disorders in ruminants (such as acidosis), leading to performance losses in dairy herds (11, 12, 23). Blood gas analysers need to be validated and compared with pioneer blood gas analysers that have been validated for precision and accuracy, as has been done for the GEM Premier 3000 (4, 10, 22, 24, 26). On the other hand, even a small deviation between the measured values can lead to significant differences despite a high correlation, which can be important for the emergency treatment of patients (29). To the best of the authors' knowledge, no study has yet addressed the validation and comparison of the Edan i15 Vet POC blood gas analyser with other reference blood gas analysers for the measurement of blood gases, electrolytes and hematocrit in lactating Holstein or other milk cow breeds. As Holsteins are one of the most important dairy breeds in the world, the main objective of the present study was to investigate the correlation, agreement and bias of Edan i15 Vet compared to Gem Premier 3000 in terms of analysis of blood gases, Na^+, K^+, hematocrit and hemoglobin in lactating Holsteins. In addition, their analytical performance was evaluated to determine correlations between blood gas parameters and postpartum time, parity and blood pH.

Material and methods

This study was conducted in accordance with the National Research Council Guide for the Use of Animals and approved by the Animal Ethics Committee of Muğla Sıtkı Koçman University, Muğla/Turkey (MUDEM-HADYEK, 23/09/2021-28/21).

Animals. One hundred and twenty-three clinically healthy Holstein cows were randomly enrolled from different dairy farms in the Aegean region of Turkey. The distribution of the study cows within one month postpartum days (PPD) was n = 29 (calving day), n = 29 (PPD 1), n = 12 (PPD 2), n = 15 (PPD 3), n = 7 (PPD 4-5), n = 10 (PPD 6-7), n = 7 (PPD 8-10), n = 5 (PPD 11-16), n = 4 (PPD 17-19) and n = 5 (PPD 22-27). Thirty-five of the 123 cows were primiparous (PRP), and the rest (n = 88) were multiparous (MUL). The cows had a dry standing period of 55-60 days. According to the information from the farms, cows were fed according to their lactation stages (dry, just before lactation, early lactation) and received *ad-libitum* water.

Blood collection and analysis of the parameters. Whole blood was collected from the coccygeal vein with a 20-gauge needle (0.9 × 38 mm) into sterile blood collection tubes (BD Vacutainer, Becton, Dickinson U.K. Limited, Berkshire, UK) without anticoagulant. Two mL of whole blood was then drawn from the tubes into 100 µL lithium heparin-containing injectors (ARD blood gas injector, ADR group) for immediate blood gas analysis. Lithium heparinised whole blood was analysed in GEM Premier 3000 (Instrumentation Laboratory Inc. Lexington MA, USA) and EDAN i15 Vet (Edan Instruments, Inc. Shenzhen, China) for pO_2 (partial pressure of oxygen), pCO_2 (partial pressure of carbon dioxide), ion concentrations of Na^+, K^+ and haematocrit (Htc) value. Both blood gas analysers measured pCO_2, Na^+ and K^+ with potentiometric sensors and pO_2 with amperometric sensors. Hct (%) was analysed using the electrical conductivity method (conductance sensors). Samples were analysed within 10 minutes of collection, carefully mixed and manually rotated until use. Both analysers were in the same room during analysis. According to the manufacturer instructions, EDAN and GEM automatically calculated the estimated values of actual HCO_3^- (actual bicarbonate ion concentration, mM), sO_2 (oxygen saturation of hemoglobin,%), TCO_2 (free and bound, total carbon dioxide, mM), BE ecf also *in vivo* BE (base excess of extracellular fluid, mM), BE B also *in vitro* BE (base excess in blood, mM) and Hb (hemoglobin concentration, g/dL) based on the formulae given below:

HCO_3^- act (GEM) = $0.031 \times pCO_2 \times 10^{(pH-6.1)}$

HCO_3^- act (EDAN) = $0.0307 \times pCO_2 \times 10^{(pH-6.105)}$

TCO_2 (GEM and EDAN) = $HCO_3^- + (0.0307 \times pCO_2)$

BE ecf (GEM and EDAN) =

$= HCO_3^- - 24.8 + (16.2 \times (pH - 7.4))$

BE B (GEM) = $(1 - 0.014 \times Hb^*) \times$

$\times (HCO_3^- - 24.8 + (1.43 \times Hb^* + 7.7) \times (pH - 7.40)$

*: estimated hemoglobin by GEM.

BE B (EDAN) = $(1 - 0.014 \times Hb^*) \times$

$\times (HCO_3^- - 24.8 + (1.43 \times Hb^* + 7.7) \times (pH - 7.40)$

*: default of 15 g/dL

Hb (GEM) = $(0.31) \times Hct\%$

Hb (EDAN) = $(34\ g/dL) \times Hct/100$

sO_2 estimated (GEM) =

$$pO_2O = 100 / \left[1 + \frac{23400}{(pO_2pp)^3 + 150 \times pO_2pp}\right]$$

According to the user manual GEM, pO_2pp is calculated using the Severinghaus formula, where BE B and e = 2.718 are used for the calculations according to the Siggaard-Aderson equation.

sO_2 estimated (EDAN) =

$$sO_2(est) = \frac{pO_2^{*3} + \alpha \times pO_2^*}{pO_2^{*3} + \alpha \times pO_2^* + \beta} \times 100$$

The EDAN user manual did not provide any further explanation on how to calculate the sO_2 value. GEM has used estimated Hb concentration when calculating BE B and sO_2, also BE ecf is calculated from GEM based on pH 7.40 with a pCO_2 40 mmHg at 37°C. Before starting the analysis of the samples with EDAN, a calibration was always performed with the appropriate calibration kits (i15 Calibrant Fluid Pack) from the manufacturer. EDAN aspirated the sample directly and required a minimum sample volume of 140 µL for analysis, with electrochemical sensors at a temperature of 37°C. The frequency of calibration of GEM was automatic at timed intervals. GEM performed one-point calibrations every 20 minutes and two-point calibrations every 2 hours. It also performed a one-point calibration after each sample analysis. GEM did not require any gas tanks, electrode membranes or third party solutions. Using 135 µL of whole blood, it analysed automatically with electrochemical sensors at a fixed electrode chamber temperature of

37°C. According to the manufacturer's instruction manual, the linearity of EDAN was satisfactory compared to the reference methods Chemistry Analysis System and Rapid Point 400 System. GEM was compared and validated with other reference blood gas analysers ‚Ciba Corning 865' and ‚Radiometer ABL725' and proved to be highly correlated and in agreement (4, 24, 26).

Statistical analysis. MedCalc software, version 2022 (MedCalc Software Ltd Acacialaan 22, Ostend, Belgium) was used to perform the statistical analyses. The significance level for all statistical tests was set at $\alpha = 0.05$. Normality of the data was checked using the Shapiro-Wilk test. Non-parametric data and some small sample sizes were analysed with the Wilcoxon test. Mean ($\bar{x}$), standard deviation (SD) and percentage ratio were presented as descriptive statistics when required. The Passing-Bablok regression equation was used to compare the test sets (17, 30). The correlation equation was expected to have a slope close to 1.0 and an intercept close to 0.0 for good agreement. Perfect agreement shows that the fitted Passing-Bablok regression obscures the 45-degree line (Y = X). Bland-Altman plots of agreement were constructed to determine the deviations and confidence intervals between the quantitative results of the two instruments (5, 13). Cohen's Kappa coefficient (7, 20) was calculated for the inter-rater reliability of the variables to observe the agreement rates (≤ 0.00 for no agreement, 0.01-0.20 for slight agreement, 0.21-0.40 for fair agreement, 0.41-0.60 for moderate agreement, 0.61-0.80 for substantial agreement, 0.81-1.00 for perfect agreement). In addition, Pearson correlation coefficients were calculated by least squares regression analysis (LSRA) for all blood parameters analysed with GEM and EDAN. LSRA was performed between blood gas parameters and postpartum times (PPT) after calving, parity and blood pH. The reference values (the lower and upper reference limits) of all blood parameters were set based on the values of 2.5-97.5% quartiles of the population for each parameter.

Results and discussion

Descriptive statistics, differences in means and reference ranges were presented in Table 1. Passing-Bablok regression analysis showed no deviation from linearity in the CUSUM test for any of the parameters ($p > 0.05$), but there were some proportional and systematic differences in the regression equations (Tab. 2). The 95% confidence intervals (CI) of the intercept included zero and the slope included 1.0 in the regression equations of pCO_2, HCO_3^-, TCO_2, BE (B), BE ecf, Hct and blood pH. However, there was no agreement in the regression equations for sO_2 and pO_2. The 95% CI of the intercept did not include zero for the Na^+ and K^+ regressions, but the slope included 1.0. For the hemoglobin regression, the 95% CI of the slope did not include 1.0. Pearson correlations were significant (Tab. 2) and were above $r = 0.90$ for K^+, HCO_3^- and pO_2 between $r = 0.80$ and 0.90 for Na^+, pCO_2, sO_2 and blood pH analysis and below $r = 0.80$ for BE (B), BE ecf, TCO_2, Hb and Hct. The agreements indicated by Cohen's kappa are slight for Hb, fair for Hct, moderate for BE (B), Na^+, TCO_2 and pH, and substantially good for pO_2, pCO_2 and K^+ (Tab. 2). Mean and total deviations (at 95% CI) by Bland-Altman plots were shown in Figures 1, 2, 3 and 4. EDAN

Tab. 1. Descriptive statistics ($\bar{x} \pm$ SD, minimum, maximum), difference of mean and reference ranges (2.5-97.5% quartiles) of blood parameters analysed by GEM and EDAN in 123 clinically healthy lactating Holstein between calving and postpartum day 27

Parameters		All cows (n = 123)	Min-Max	Difference of mean (EDAN-GEM) (%)	Reference values (low/high)
Na^+ (mM)	GEM	136.86 ± 4.69	124-146	1.44	125/145
	EDAN	138.83 ± 4.41*	122-150		127/146
K^+ (mM)	GEM	4.09 ± 0.39	3.1-5.3	2.44	3.3/4.9
	EDAN	4.19 ± 0.38*	3.4-5.3		3.5/5.0
pCO_2 (mmHg)	GEM	40.64 ± 5.53	25-59	–1.62	32/52
	EDAN	39.98 ± 5.84*	25-55.9		25/51
pO_2 (mmHg)	GEM	40.24 ± 22.51	18-138	3.03	20/131
	EDAN	41.46 ± 21.04*	22-140		25/116
HCO_3^- (mM)	GEM	28.57 ± 3.67	17.8-38.4	–5.28	22.8/37.7
	EDAN	27.06 ± 3.47*	17.9-36.3		21.1/35.1
TCO_2 (mmHg)	GEM	29.65 ± 4.21	9.6-39.8	–7.25	23.8/39.3
	EDAN	27.50 ± 3.96*	14.9-37.0		17.6/35.0
BE ecf (mM)	GEM	4.93 ± 4.81	(–6.0)-15.5	–38.54	(–1.8)/14.8
	EDAN	3.03 ± 3.68*	(–6.1)-12.0		(–3.4)/11.3
BE B (mM)	GEM	4.29 ± 3.49	5.1-14.0	–25.17	(–1.6)/12.9
	EDAN	3.21 ± 5.51*	(–4.9)-10.5		(–2.9)/10.5
sSO_2 (%)	GEM	68.66 ± 17.19	28.0-99.0	2.67	36/99
	EDAN	70.49 ± 15.42*	38.0-99.0		43/99
Hct (%)	GEM	26.93 ± 3.51	15.0-36.0	10.40	19/35
	EDAN	29.73 ± 4.36*	14.0-36.0		19/38
Hb (g/dL)	GEM	8.34 ± 1.06	4.7-11.2	21.22	5.9/10.9
	EDAN	10.11 ± 1.15*	4.7-16.4		6.6/13.1
Blood pH	GEM	7.46 ± 0.04	7.36-7.56	–0.13	7.38/7.54
	EDAN	7.45 ± 0.04*	7.37-7.56		7.37/7.53

Explanation: * – $p < 0.001$ significantly different from GEM in the rows for the respective parameters (by Wilcoxon test); pO_2 – partial pressure of oxygen; pCO_2 – partial pressure of carbon dioxide; TCO_2 – total carbon dioxide; BE ecf – Base excess of extracellular fluid; BE B – base excess in the blood; sO_2 – an estimation of hemoglobin oxygen saturation; Hct – hematocrit; Hb – hemoglobin

yielded 2.4% and 3.0% higher and –5.3% lower mean values (bias) for K^+, pO_2 and HCO_3^-, respectively, compared to GEM (Fig. 1 and 2, Tab. 1). The majority of the samples were within the confidence intervals of the Bland-Altman plots (95.9% for HCO_3^-, 96.7% for K^+, 94.3% for pO_2). EDAN provided 1.4%, –1.6%

Tab. 2. Regression equation, systematic, proportional and random differences by Passing-Bablok (PB) regression equation, Cohen's Kappa coefficients and correlation coefficient by least squares regression between EDAN and GEM blood gas devices for the analysis of blood gases, electrolytes, hematocrit and hemoglobin in 123 lactating Holsteins

Parameters	Regression equation by PB	Systematic differences	Proportional differences	Random differences	Cusum linearity test	Cohen's coefficient	CC
	y =	Intercept 95% CI	Slope 95% CI	RSD (± 0.96)	p =	Kappa (95% CI)	r
Na^+ (mM)	2.000 + 1.000x	2.000/2.600	0.824/1.000	18.83 ± 3.69	0.68	0.50 (0.42/0.59)	0.83
K^+ (mM)	0.100 + 1.000x	0.100/0.100	1.000/1.000	0.08 ± 0.15	0.27	0.72 (0.67/0.78)	0.96
pCO_2 (mmHg)	–1.317 + 1.017x	(–6.133)/2.225	0.925/1.133	22.05 ± 4.32	0.81	0.64 (0.58/0.79)	0.85
pO_2 (mmHg)	6.250 + 0.875x	4.308/7.667	0.833/0.923	29.30 ± 5.74	0.69	0.77 (0.72/0.82)	0.98
HCO_3^- (mM)*	–0.829 + 0.978x	(–2.810)/1.260	0.900/1.050	10.32 ± 2.02	0.92	0.60 (0.54/0.66)	0.92
TCO_2 (mmHg)*	–1.400 + 1.000x	(–4.888)/1.181	0.909/1.111	27.40 ± 5.37	0.84	0.49 (0.40/0.57)	0.57
BE (B) (mM)*	–1.385 + 0.954x	(–1.735)/0.987	0.875/1.034	24.52 ± 4.81	0.78	0.60 (0.54/0.66)	0.69
BE ecf (mM)*	–1.216 + 0.968x	(–1.638)/0.926	0.886/1.052	30.52 ± 5.98	0.85	0.58 (0.52/0.65)	0.64
sSO_2 (%)*	13.125 + 0.844x	9.200/16.200	0.800/0.900	56.02 ± 10.98	0.37	0.60 (0.54/0.66)	0.88
Hematocrit (%)	–2.800 + 1.200x	(–6.333)/3.000	1.000/1.333	20.14 ± 3.95	0.52	0.33 (0.26/0.41)	0.73
Hemoglobin (g/dL)*	–1.100 + 1.333x	(–2.624)/0.060	1.200/1.520	0.63 ± 1.24	0.95	0.13 (0.08/0.18)	0.74
Blood pH	–0.010 + 1.000x	(–0.010)/0.612	0.917/1.000	0.02 ± 0.03	0.71	0.60 (0.53/0.68)	0.86

Explanation: BE ecf – base excess of extracellular fluid; BE B – base excess in the blood; * – calculated parameters by each device; RSD – residual standard deviation (95% confidence intervals); CC – correlation coefficients by the least squares regression analysis, CI – confidence intervals; Cusum linearity test (cumulative sum linearity test): P > 0.05 no significant deviation from the linearity

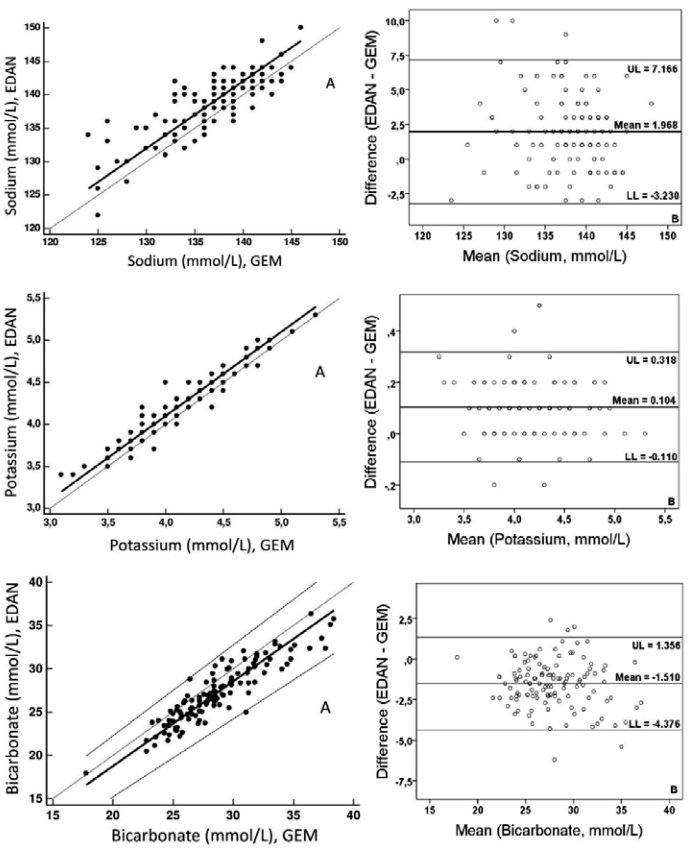

Fig. 1. Passing-Bablok regression analysis (A) and Bland-Altman plots of agreement (B) between EDAN and GEM blood gas devices for the analysis of sodium, potassium and bicarbonate (95% confidence intervals) in 123 lactating Holstein
Explanations: UL – upper limit; LL – lower limit

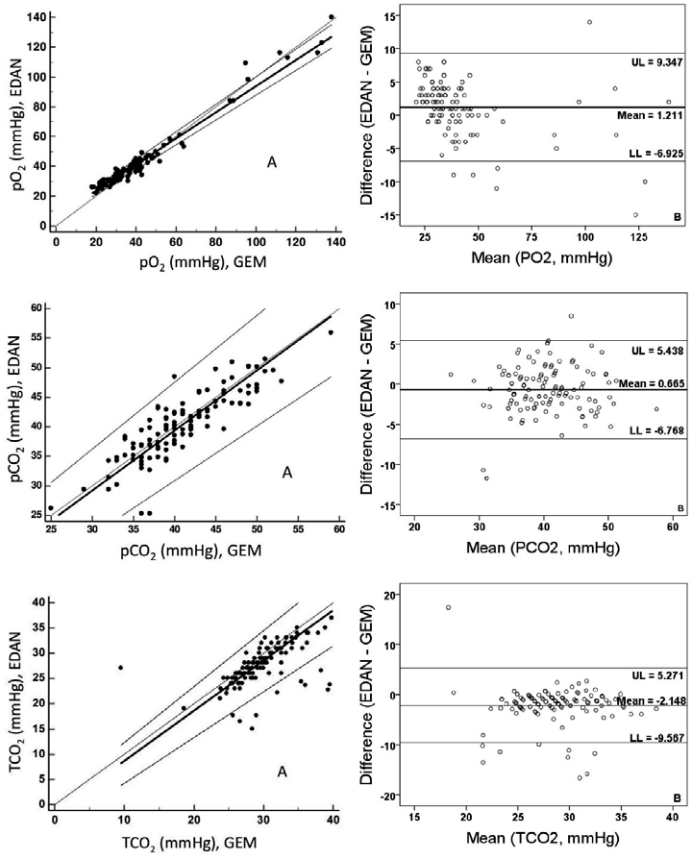

Fig. 2. Passing-Bablok regression analysis (A) and Bland-Altman plots of agreement (B) between EDAN and GEM blood gas devices for the analysis of pO2, pCO_2 and TCO_2 (95% confidence intervals) in 123 lactating Holsteins
Explanations: UL – upper limit; LL – lower limit

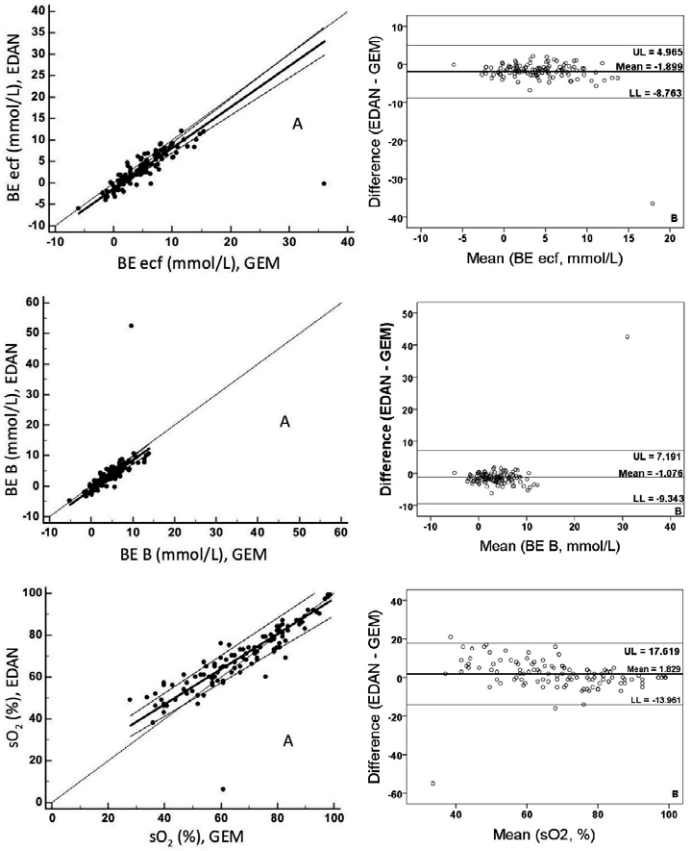

Fig. 3. Passing-Bablok regression analysis (A) and Bland-Altman plots of agreement (B) between EDAN and GEM blood gas devices for the analysis of base excess extracellular fluid (BE ecf), base excess blood (BE B) and estimated O_2 saturation of hemoglobin (sO_2) (95% confidence intervals) in 123 lactating Holstein

Explanations: UL – upper limit; LL – lower limit

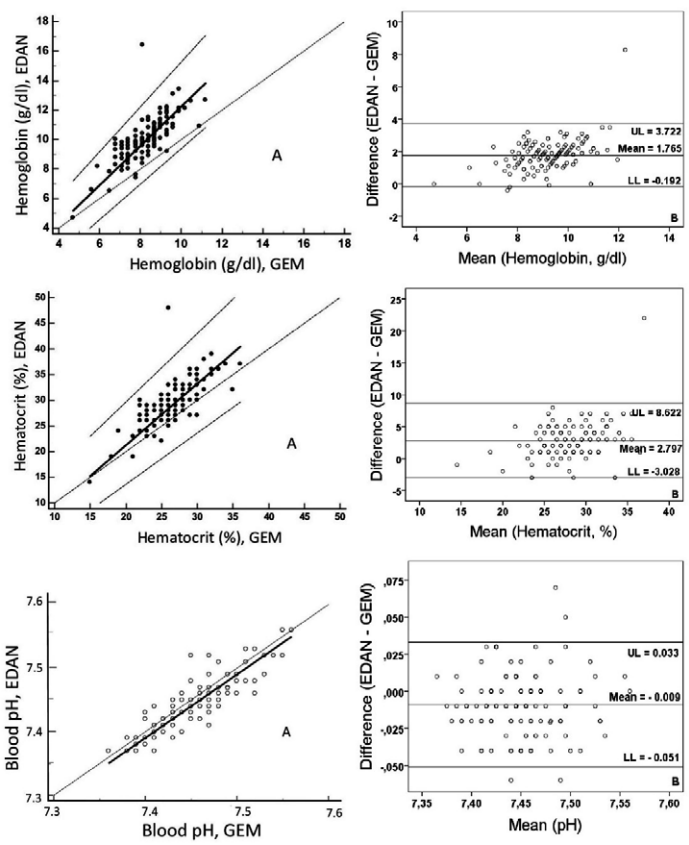

Fig. 4. Passing-Bablok regression analysis (A) and Bland-Altman plots of agreement (B) between EDAN and GEM blood gas devices for the analysis of hematocrit, hemoglobin and blood pH in 123 lactating Holstein

Explanations: Confidence intervals 95%: (UL – upper limit, LL – lower limit)

and 2.7% of the mean values (bias) for Na^+, pCO_2 and sO_2 compared to GEM and more than 97% of the samples were within the CI of the Bland-Altman plots (Figs. 1, 2, 3, Tab. 1). A negative bias was observed in the calculation of TCO_2, BE ecf and BE B, resulting in –7.3, –38.5 and –25.3% lower mean values of EDAN compared to GEM (Tab. 1, Figs. 2 and 3), although the majority of samples (BE ecf: 99.2%, TCO_2: 91.9%, BE B: 99.2%) were within the 95% confidence intervals of the Bland-Altman agreement plots (Figs. 2 and 3). Most samples were within the 95% CI in the Bland-Altman plots (98.4% for Hb and 99.2% for Hct), but the mean values provided by EDAN were 10.4 and 21.2% higher (bias) for Hb and Hct, respectively, than for GEM (Fig. 4 and Tab. 1). A small negative mean deviation (–0.1%) and an overall bias of –0.13 by Bland-Altman plots and an acceptable linearity equation were observed for EDAN compared to GEM, indicating good agreement in blood pH analysis (Tab. 1, Fig. 4). Parity did not correlate with blood gases, electrolytes, Hb and Hct, as confirmed by both GEM and EDAN ($p > 0.05$). Significant negative (Hb, Hct) and positive (pCO_2, HCO_3^-, BE ecf and BE B) correlations were found between PPT and blood gas parameters, which was confirmed by both instruments. EDAN could not establish a correlation between PPT and TCO_2 ($p > 0.05$), but GEM found a significant positive correlation. Blood pH correlated negatively ($p < 0.05$) with pCO_2 and K^+ values and it correlated positively ($p < 0.05$) with pO_2, BE ecf, BE B, HCO_3^-, sO_2 and TCO_2, which was confirmed by both blood gas instruments. However, GEM did not find a significant negative correlation ($p > 0.05$) between blood pH and Na^+ levels, while EDAN showed a significant negative correlation.

The POC analysers may be more practical for rapid analysis of blood gases and electrolytes in lactating cattle and calves on dairy farms. However, the accuracy of these analysers needs to be tested with already validated blood gas analysers or reference methods. GEM has been validated for precision and accuracy with other blood gas analysers (4, 24, 26) and hematocrit analyser (24), as well as with standard gold methods or performance measures in terms of mean downtime (10). However, borderline results were obtained for glucose, lactate and potassium analysis at certain concentrations (26). The present study showed that the highest correlations between EDAN and GEM were obtained for the analysis of K^+, pO_2 and HCO_3^-, but high correlation does not always imply an agreement

between methods (13, 17), as shown in the presented study for pO_2. Zatloukal et al. (29) also confirmed this statement by Hb analysis with GEM and HemoCue 201, which showed a high correlation but unsatisfactory agreement. In addition, linearity acceptance, agreement and bias between GEM and EDAN were tested using Passing-Bablok analysis (17, 30), Bland-Altman agreement plots (5, 13) and Chen's Kappa coefficient (7, 20). The Cusum linearity test did not reject the linearity between EDAN and GEM, so they are comparable (30).

A low and acceptable mean bias indicates a good agreement between GEM and EDAN for the analysis of blood pH. Others (3) reported a much higher mean bias (0.06) in the comparison of blood gas analysers. Similarly, substantial good agreements were observed in the analysis of pCO_2 and HCO_3^- indicating less than or about 5% in the mean. The 45-degree line was obscured by the regression of pCO_2 with acceptably small systematic and proportional differences. The resulting Kappa was classified as moderate agreement for the medical parameters because they ranged between 0.60 and 0.79 (20). A systematic error was observed on the regression equations for the analysis of K^+ and Na^+, but the slopes included 1.0. The resulting bias of about 2.5% in the mean, the moderate agreement according to Cohen's Kappa (20), less than 0.5 mmol/L total error (27) suggested an acceptable agreement in the K^+ analysis between EDAN and GEM. However, the agreement was weak for Na^+ analysis on Cohen's Kappa according to McHung (20). In contrast, the less than 1.5% positive bias in the mean value looked small and acceptable for the Na^+ analysis from a clinical point of view, but the total error of 10.3 mmol/L was higher than the allowable value defined for the Na^+ analysis (4.0 mmol/L) (27). In addition, Zulle (29) reported good agreement between regression equations, indicating for a systematic error but no proportional error. McHugh (20) pointed out that kappa has strengths and limitations and should therefore be interpreted carefully, especially in medical sciences. This can clarify the ambitious coefficient of Cohen's Kappa, although errors were observed by the Passing-Bablok regression equation. Passing and Bablok regression equations have shown a constant and proportional errors for the pO_2 and sO_2 analysis indicating no agreement between GEM and EDAN. Interestingly, Cohen's kappa coefficient indicated a substantial good agreement for pO_2 and moderate agreement for sO_2 analysis. However, these coefficients were classified as moderate by McHung because of the medical characteristics of the parameters (20). In addition, a positive bias of about 3% in the mean but high total errors for the sO_2 and pO_2 analysis appeared to be outside the allowable biological variation for pO_2 (10% group variation) (27). A moderate correlation ($r = 0.79$) was previously reported between GEM and Ciba Corning 865 (24) in the analysis of K^+. GEM has been used to test blood gases and electrolytes in calves with diarrhea and significant differences have been found between healthy and diseased animals (2). However, the correlation of GEM Premier 3500 was compared to other devices to analyse ionized calcium in cows only (25). Furthermore, negative correlations were found by EDAN between blood pH and blood pCO_2, K^+ and Na^+ values confirmed by GEM except for the insignificant negative correlation between blood pH and Na^+. The reason for this might be the weak kappa coefficient according to McHung (20) and systematic error suggested by Passing-Bablok regression equation in the analysis of Na^+. A positive correlation between blood pH and pO_2, sO_2, BE ecf, BE B, TCO_2 and HCO_3^- were confirmed both by EDAN and GEM. Passing-Bablok regression equation indicated a good agreement between EDAN and GEM concerning the analysis of TCO_2, BE ecf and BE B because 95% CI of intercept and slope included zero and 1.0 respectively, but the resulting kappa coefficients were weak for TCO_2 and BE ecf and moderate for BE B. The high biases in mean and total error provided by Bland-Altman plots did not allow the clinical acceptance of this weak to moderate agreement between two analysers because BE determines the treatment protocol of blood misbalanced acid-base status directly (11, 12, 23) although allowable total errors are in the range of 4.8 and 85% for the analysis of TCO_2 and BE respectively. Moreover, both devices confirmed a significant positive correlation between PPT and blood pCO_2, TCO_2, BE ecf, BE B and HCO_3^- and no correlation with K^+, Na^+ and sO_2. PPT correlated negatively with Hct and Hb values, implying that the values of Hb and Hct decreased with the time after calving which is consistent with other studies (28). As expected, blood pH did not correlate with Hct and Hb values. Both GEM and EDAN agreed with these results. However, a weak or non-agreement in Kappa and high positive bias in mean of Hb and Hct between GEM and EDAN were not acceptable from a clinical point of view and could jeopardise treatment success. Therefore, they cannot be used interchangeably. The total allowable errors was given as 6% for the Hct analysis (27) which was in line with the suggestions of the present study.

Regression analyses and coefficients of variations between GEM and other reference blood gas analysers were performed only for directly measured parameters (4, 24, 26). Linearity was acceptable and comparative results showed good agreement with correlation coefficients between 0.91 and 0.99 (4). In the present study, not only directly measured parameters were analysed, but also estimated parameters based on a fixed calculation formula. The reason for the low to moderate linear correlations of the estimated parameters could be the small differences in the fixed calculation formulas of the respective analysers. This applied in particular to the calculations of the following parameters Hb, TCO_2, BE ecf and BE B. Correct

calculations of estimated parameters such as BE ecf, BE B, HCO_3^- and TCO_2 are important during acidosis treatment in lactating cows or calves to balance the acid-base deficit. Currently, the theory of strong ion difference has proven to be more relevant and can provide more detailed information about the acid-base status in comparison to the traditional parameters BE and HCO_3^- in blood, especially in cases of subclinical acid-base disorders (11). The blood pH of ruminants depends on the relative concentrations of bases, acids and buffers (23). BE is normally present in the blood, but exposure to acids such as in acidosis can decrease BE and consequently can overcome the buffering capacity of HCO_3^- (12). The mean concentrations of BE, TCO_2 and HCO_3^- in the present study were similar to the results of other studies (6) where the results from jugular venous blood in Holstein have been presented. Bajcsy et al. (3) reported significantly different mean values of pH, pCO_2 and pO_2 but not for bicarbonate and BE in blood collected from jugular, coccygeal and mammary veins of Holstein cows. Reports by Bajcsy et al. (3) showed that the mean pCO_2 of blood from the coccygeal vein and jugular vein were higher and the values of pO_2 and pH from the coccygeal vein were lower than in the present study. However, BE and bicarbonate values were similar to the ones in the study of Bajcsy et al. (3). So were also the values of blood pO_2, BE, bicarbonate and pH from the jugular vein. Bajcsy et al. (3) found a large difference in blood pH between the blood gas analysers (0.06 units). The present study also found a difference between GEM and EDAN, but it was much smaller. The reason for the small difference between the mean values or the reference ranges could be the different handling or the patient temperature entered into the analyser (6, 11, 12) or also the calculation formula of the estimated parameters or the place of blood collection (3). Nevertheless, it can be stated that EDAN and GEM correspond in the blood pH analysis according to the values reported in the literature (3).The mean data of the present study were within the reference ranges for pH, pCO_2, HCO_3^- and BE ecf reported by others (16) in arterial blood of the Holstein Friesian. However, the lowest limit of arterial pO_2 was higher than in the present study. The mean sO_2 value in the present study was higher than in the study by Guzelbektas et al. (15) and lower than in the study by Cingi et al. (6). When comparing the pH values of clinically healthy Holstein cows from these two studies, it is noticeable that they are lower than those in the present study. The oxygen saturation rate of hemoglobin (sO_2) indicates the highest O_2 availability since most of the blood oxygen is bound to hemoglobin, only a small part circulates freely in the blood. It has been reported that low pH-induced separation of O_2 from hemoglobin results in reduced sO_2 (6, 14).

The mean values of venous pO_2, K^+, HCO_3^-, BE and pH that were reported by others (15) using a different blood gas analyser were slightly lower than the results of the present study. But values of pCO_2, Na^+ and Hb concentration in other studies (15) were much closer to the values of EDAN. Hct is a directly analyzed parameter by GEM and EDAN, but their correlation was border-lined. Zatloukal et al. (29) compared the linearity and accuracy of GEM with a laboratory reference method for Hb analysis and they found significant coefficients of determination and similar mean values of Hb. However, the authors have concluded that despite a high correlation with laboratory measurements, the absolute accuracy of analysers tested was low due to clinical treatment concerns. Wholt et al. (28) reported an average Hct value of 40 to 30.8% tested by the micro-capillary Hct method in lactating Holsteins between calving and PPD 28. They observed higher Hct at calving and in the first days after calving, which was consistent with the negative correlation of Hct and Hb with PPT in the present study. However, Hct values in the first days after calving were higher than the values of GEM and EDAN. Jones et al. (18) found a significant positive effect of high milk production on Hct and Hb, but this was not the case in the present study. The mean values of Hct and Hb were reported as 31.6% and 11.9 g/dL respectively in lactating Holsteins. The authors (18) also reported the lowest and highest Hct and Hb values from different studies as 30.3-33.7% and 10.0-12.1 g/dL respectively. These results were close to the mean values of EDAN, but higher than GEM.

In conclusion, EDAN can be used interchangeably with GEM for the analysis of blood pH, K^+, pCO_2 and HCO_3^- as they have acceptable agreement. Yet both analysers did not have acceptable agreement for other calculated parameters such as TCO_2, BE ecf, BE B, Hb, sO_2 and for measured parameters as such Na^+, Hct and pO_2. Therefore, the two analysers cannot be used interchangeably. The mean values and reference ranges of the parameters for GEM and EDAN more or less corresponded to the values given in the literature. Furthermore, both analysers must be tested on sick animals, which could shift the respective blood parameters out of reference ranges. The two devices need to be checked with a reference laboratory method for Hct (microhematocrit method) and Hb analysis to allow more accurate comparison in lactating Holsteins, although the Hct and Hb values from EDAN were more or less in agreement with previously published data in lactating Holsteins.

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Corresponding author: Abdülkerim Deniz, Assoc. Prof., Free researcher for clinical biochemistry, Nisbetiye Mah. Beşiktaş, Istanbul, Turkey; e-mail: ad.deniz68@gmail.com