THESIS

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The EPOC Blood Analysis System: performance evaluation of various analytes. A retrospective pilot study

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Na^+	sodium
K^+	potassium
Cl^-	chloride
Ca^{2+}	calcium
Hb	hemoglobin
Ht	hematocrit
G	glucose
Cre	creatinine
POCT	point-of-care testing
EPOC	enterprise point of care
UVMB	University of Veterinary Medicine, Budapest
SOP	standard operating procedures
CV	coefficient of variation
TEa	total allowable error
r	correlation coefficient
ICU	intensive care unit
BGE	Blood Gas and Electrolyte
BGEM	Blood Gas Electrolyte and Metabolites
pCO_2	partial pressure of carbon dioxide
pO_2	partial oxygen pressure
$cTCO_2$	total carbon dioxide
BE	base excess
cSO_2	oxygen saturation

$cHCO_3$-	bicarbonate
BUN	blood urea nitrogen
ISE	ion selective membrane electrode
RBC	red blood cell
MCV	mean corpuscular volume
ATP	adenosine triphosphate
ADP	adenosine diphosphate
EDTA	ethylenediamine-tetra-acetic acid
CI	confidence interval
CLIA	clinical laboratory improvement amendments
ASVCP	American Society for Veterinary Clinical Pathology
SD	standard deviation
PCV	packed cell volume
TP	total protein
IRIS	International Renal Interest Society
CKD	chronic kidney disease
AKI	acute kidney injury
nm	nanometer
µm	micrometer
µl	microliter
µmol/L	micromole per liter
mg/dL	milligram per deciliter
g/dL	gram per deciliter
g/L	gram per liter

1. Introduction

Acid-base measurement is a cornerstone of intensive, care providing data that may guide therapeutic interventions. Apart from acid-base data modern devices also measure several parameters with which clinicians may narrow down their diagnosis of acid-base disorders and monitor conditions which alter these parameters. Frequently measured additional parameters are ions such as sodium (Na+), potassium (K+), chloride (Cl-) and calcium (Ca2+); hemoglobin (Hb) and hematocrit (Ht); glucose (G), lactate and creatinine (Cre), all of which aid the diagnosis and monitoring of disturbances leading to acid-base disorders. Advancements in technology allow the use of point-of-care (POC) devices which leads to an improved turnaround time and therefore improved patient care.

Laboratory measurements are affected by imprecision and bias. Providing accurate results within predefined acceptability criteria is a desirable goal of the veterinary laboratory. Different instruments using varying methods to measure the same analyte may be affected by the aforementioned factors in an altering fashion. Method comparison studies are carried out to compare measurements on different devices and assess acceptability and interchangeability, which determines whether the use of one device or method can be fully accepted instead of another.

Previously it has been demonstrated that creatinine measured by the EPOC acid base analyzer shows significant bias within its reference range [1]. This bias appeared to be linear. In the present study, a retrospective investigation was carried out to determine how creatinine as well as other analytes (namely sodium, potassium, glucose, hemoglobin, and hematocrit), perform, compared with a reference method throughout clinically significant ranges. These analytes are frequently measured in parallel in the Teaching Hospital and at the Clinical Laboratory of the University of Veterinary Medicine Budapest (UVMB). Due to the lack of data on feline patients, only canine samples were investigated.

2. Literature review

2.1 Method comparison studies

As soon as a new instrument is installed or a newly developed system is launched in the veterinary laboratory, it is important for practitioners to find out whether it is a good alternative to the method in use. Method comparison studies are carried out to assess the comparability of the new method [2, 3]. By assessing the new method, insights can be given into its clinical usefulness. Other considerations include cost, availability of reagents, sampling requirements, ease of use, and turnaround time. Whether the usage of the new system is appropriate or not is judged by personal evaluation, statistics, and objective standards to help in judging its analysis process [3].

Initially, method comparison studies summarized the results in tables without any statistical analysis, such as in probably the first article in the veterinary field by McCarthy published in 1965 on four different methods of urea management. This article mostly focused on the practicalities of the measurement [4]. Statistical methods were introduced later, although the theoretical background (linear regression in most cases) was well-known before. Passing and Bablok specifically wrote about linear regression procedures for method comparison studies in 1983 [5]. Bland and Altman further expanded the possibilities of statistical investigation in 1986 [6] in an article that is still one of the most cited papers of all time [7].

With the ever-expanding analytical armament of the veterinary laboratory, the need for a standardized approach to method comparison appeared. An influential article was published by Jensen and Kjellgrad-Hansen in 2006 which summarized the theoretical background and practicalities of method comparison for veterinary clinical pathologists and other stakeholders [3].

2.1.1 Errors

What needs to be taken into account is that whenever laboratory methods are used, errors are inherently present. It is relevant to try to keep those errors at a minimum by employing educated staff, using certified methods and sticking to their standard operating procedures (SOP), running quality checks, and knowing about the different errors and why they occur [3].

Erroneous test results can have different causes and can be classified in the following way:

1. Preanalytical, when for example the wrong method was used, a different patient was analyzed or the sample wasn't handled correctly
2. Total analytical error (random and systematic)
3. Postanalytical when there was a wrong numbering or misspelling, a mistake in the transcription, etc. [3]

Analytical errors affect the measurement and can be classified as random errors and systematic errors. Random error can be viewed as the precision of the result. It is independent of the true value of the analyte. Imprecision is the quantitative opposite of it and is determined as a standard deviation or coefficient of variation of the measurement results [3]. Systematic error (also known as bias or inaccuracy) is the difference between the true value and the result of the measurement (theoretically the mean of the differences from the true value if infinite measurements are taken from the same sample). In reality, it is impossible to have infinite numbers of measurements, so fewer measurements are done. Having a true value is also impossible as it would have to be from a perfect measurement. Instead, a value that was determined by a reference method is used. The systematic error can be further divided into constant (approximated as the average differences between the two methods) and proportional (the differences between the two methods are proportional to the measurements) systematic error [3]. All of the described types of errors are shown in Figure 1.

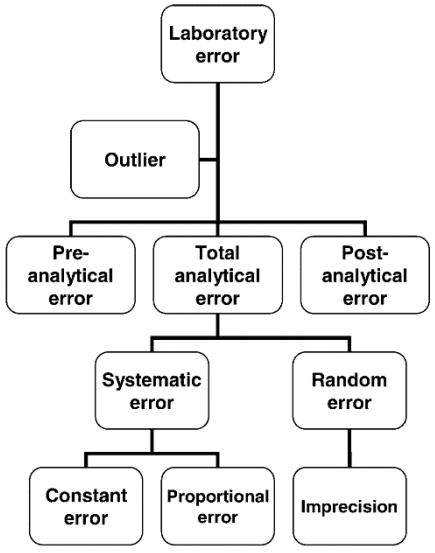

Figure 1: Types of errors adapted from [2]

2.1.2 Protocol for method comparison

Different steps need to be taken into consideration when performing a method comparison study [2, 3]. Initially, the motivation for the research has to be clarified and one has to become acquainted with the new procedure. The second step is the assessment of the possible sources of differences between the methods (reaction principles, sample type, possible interferences, and other sources of error).

The acceptability of the new method is assessed based on estimates of random error for both methods. Approximation of random error (defined as the coefficient of variation or CV) for both methods is desirable and can be used to assess the similarity of the measurement. The use of published data is also possible – often the manual of the reagents contains such data provided by the manufacturer. However, these should be used precautiously since the circumstances of the measurements may be different than the intended use [3].

As an alternative to the previous possibility the total allowable error (TEa), which encompasses bias, random error, and biological variation, can be used [2, 3]. TEa is frequently used as an analytical quality requirement in laboratories worldwide. TEa values are available in the published literature and can be used as a basis for acceptability [8, 9]. The acceptance limits to assess the interchangeability of the two methods can be based on the inherent imprecision of both methods or analytical quality specifications such as the TEa. The first mentioned uses the formula $\sqrt{CV^2_{Method1} + CV^2_{Method2}}$. The use of analytical quality specifications acquired by analyzing medical requirements in certain clinical circumstances is uncommon in veterinary medicine [2, 3].

After the theoretical phase is done, the patient samples can be measured with both techniques. Typically at least 40 samples should be used during desirably a minimum of 5 days of measuring. Replicate analysis is recommended to find outliers. As soon as the data is ready, it needs to be analyzed – a helpful way is using graphs/plots. A simple plot can be used where the results from the new method will be put on the y-axis as opposed to the older one on the x-axis, a line of identity where y=x shows the behavior of the results. Alternatively, a Bland-Altman plot also called a “difference plot” can be done [2, 3]. Here the difference of the methods (A-B) will be plotted on the y-axis and the mean of the differences (A+B)/2 on the x-axis. This way facilitates finding out errors and biases, outliers and also shows if for example there is a rise in the difference of the methods for higher values [10]. Whenever the results can be seen around the line of zero difference, the two methods

show the same results. A paired t-test can be performed to prove or disprove this theory [2, 3]. If disagreement between the methods can be seen, it can be shown by calculating the bias, approximated by the mean difference and the standard deviation of the differences. In case of a persistent bias, it can be corrected by deducting the mean difference from the new method. The mass of the differences should lie between the mean difference – 2x standard deviation of the differences and the mean difference + 2x standard deviation of the differences. If it is a Gaussian distribution, 95% of those differences will be within this range [6].

Determining the correlation coefficient "r" is often performed. It does not measure the agreement between the two methods, but the strength of correlation between two values [6]. Even though, it is still used to decide on the right regression analysis. If it is >0.99 (for data with a broader range) or >0.975 (for data with a smaller range), simple linear regression is a good way to display proportional (intercept shows remarkable difference from 0) and constant (slope differs from 1) error. On the other hand, if r is <0.975 or 0.99, extra data might be required or another form of regression analysis needs to be done – namely Passing-Bablok or Deming regression. The Passing-Bablok regression allows using extreme values and inaccuracy which does not have to be constant. Both of the compared methods might be measured with error in the case of the Deming regression [2, 3].

To decide on acceptability based on preset analytical quality specifications the TEa is used. It is necessary to determine the total error, which can be calculated by adding the systematic error and the random error together and then comparing it with the wanted TEa. The total error needs to be less than the TEa for the new method to be accepted [2, 3].

3. Aims

This work aims to determine the agreement between the EPOC analyzer's results and those obtained from reference analyzers. The parameters investigated were hemoglobin, hematocrit, sodium, potassium, glucose, and creatinine.

4. EPOC and reference methods: possible interferences

4.1 The EPOC analyzer and the reference methods

EPOC

Measurement results from the “Enterprise point of care blood analysis system”, short EPOC, which is in use in the intensive care unit (ICU) of the University of Veterinary Medicine in Budapest, are compared to reference methods used in the clinical laboratory of UVMB in the present study. EPOC legally belongs to Epocal Inc. located in Ottawa, Canada, and is a trademark of Siemens Healthcare Diagnostics Inc. [11]. Since 2006 this method has been accepted in the United States by the authority for Food and Drug Administration [12] and showed good results in accuracy testing when compared with similar devices [12,13]. Calcium-equilibrated lithium or NA-heparin blood collection syringes should be used for sampling [11].

The EPOC analyzer, which can easily be carried around, consists of three parts: EPOC Reader, Host, and Test Card [11].

The Reader, which gets its energy from batteries, has an opening to insert the Test Card which will be identified with the help of a barcode scanner and read during the blood analysis. Signal lights make it easy for the utilizer to see the progress of the ongoing test. Electrical signals will be captured by the test card sensors and the measurement outcome will wirelessly be transferred to the EPOC Host [11].

The portable small computer, also called the Host, is equipped with the EPOC host software and connects to the Reader via Bluetooth. From there it receives the sensor data and can work out analytical values from it. This part of the device is also where the user can find the test results as they will be shown on its screen [11].

An EPOC Test Card is a small non-reusable instrument with an opening to put in the blood sample. An arrangement of sensors can be found on a sensor module and calibration fluid is inside a closed reservoir which will pass over sensors within the card during the calibration process. Electrical signals are emitted by the Test Card in accordance with the concentrations in the sample compounds. Among expiration dates and production numbers, different card types can be determined through individual barcodes [11].

The two Test Cards available for the EPOC analyzer are the BGE (Blood Gas and Electrolyte) and BGEM (Blood Gas Electrolyte and Metabolites) types. The following

parameters are measured when using the EPOC BGE Test Card: sodium (Na+), ionized calcium (Ca2+), potassium (K+), pH, partial pressure of carbon dioxide (pCO_2), partial oxygen pressure (pO_2) and hematocrit (Ht). Some other values are calculated: total carbon dioxide ($cTCO_2$), base excess (BE), oxygen saturation (cSO_2), bicarbonate ($cHCO_3$-), and hemoglobin (Hb) [14]. All of the listed analytes included in the BGE Test Card are the same in the BGEM card. Additionally to those mentioned above, lactate, glucose, and creatinine are measured [11, 14].

The test card uses different sensor measurement methods for the various analytes:

1. Potentiometry for sodium, potassium, chloride, BUN (blood urea nitrogen)/Urea, pH, ionized calcium and pCO_2
2. Conductimetry for hematocrit
3. Amperometry for pO_2, glucose, lactate, and creatinine [11]

Measuring creatinine works on the basis of an enzymatic reaction, where the hydrolyzation from creatinase to creatine is the first step [15]. Amperometry for creatinine measurement according to Siemens functions the following way: “Each creatinine sensor is a three-layer enzyme electrode comprising a first immobilized enzyme creatinine-conversion underlayer coated onto a gold electrode, a second immobilized enzyme creatine screening layer, and a third diffusion barrier layer” [16].

Glucose is also measured by amperometry. “The sensor comprises an immobilized enzyme first layer coated onto a gold electrode of the electrode module, with a diffusion barrier second layer” [11]. Glucose oxidase is used to turn glucose into hydrogen peroxide and an amperometric sensor detects it by redox mediated reduction. The potentiometry measuring sodium and potassium uses an ion-selective membrane electrode (ISE) and their concentration is acquired from the potential that was measured using the Nernst equation [11].

Alternating current conductometry using two gold electrodes measures hematocrit [11].

Reference method

The chosen reference methods were those in use at the clinical laboratory at the UVMB. The Advia 2120i is a benchtop high-throughput hematology analyzer used widely in reference laboratories. It utilizes flow cytometric methods to determine cell counts in biological samples including blood. Its software enables the analysis of the blood of multiple species.

Hemoglobin is measured with a cyanide-free colorimetric method. Adding sodium lauryl sulfate turns hemoglobin into a sulfated derivative and light absorption measurement is done at 564 nm [17]. The analyzer calculates the hematocrit by multiplying the red blood cell (RBC) number by mean corpuscular volume (MCV) which is the mean value of the red blood cell volume histogram [18].

The Olympus AU400 is an automated clinical chemistry analyzer capable of spectrophotometric as well as turbidimetric measurements and measurements carried out on ion-selective electrodes alike. The former is used in the analysis of routine clinical chemistry parameters; the latter is used for the analysis of monovalent ions. [19]. The amount of sample varies from 2-50 µl for spectrophotometric methods and from 20-25µl for ion-selective electrode measurement on serum and urine. Potassium and sodium are measured with an ion-selective electrode. Creatinine is measured with the so-called Jaffe method [19]. In a non-acidic environment the creatinine-picrate complex forms after the reaction of creatinine with alkaline picrate [15, 20]. It is red-orangey in color and is also referred to as the "Janovski" complex [20]. Spectrophotometry is used at 500 nm to measure the changes in absorbance which are caused by the forming of the complex as it is in proportion to the real amount of creatinine in the sample [15, 20, 21]. Glucose is measured with hexokinase (the so-called glucose oxidase-peroxidase method) [19]. Hexokinase helps phosphorylate glucose with adenosine triphosphate (ATP) to produce glucose-6-phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate is further oxidized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase. The change in absorbance that is determined spectrophotometrically measures the glucose-6-phosphate formed from the glucose in the sample [22, 23].

Measurement methods of the EPOC analyzer and reference methods concerning the present study are presented in Table 1.

Table 1: : measurement methods of chosen analytes on the EPOC system and the reference analyzers. TEa values for the reference methods are given in brackets.1: Olympus AU400, 2: Advia 2120i, adapted from [8, 9, 11, 17–19]

	Potassium	Sodium	Glucose	Creatinine	Hematocrit	Hemoglobin
EPOC	direct ISE, potentiometric	direct ISE, potentiometric	Glucose oxidase, ampero-metric	Enzymatic, ampero-metric	Conducto-metric	Calculated from Ht
Reference method	indirect ISE, potentiometric (5 %)[1]	indirect ISE, potentiometric (5 %)[1]	Glucose oxidase-peroxidase, enzymatic colorimetric endpoint (10 %)[1]	Jaffe method, colorimetric kinetic (15 %)[1]	Calculated from red blood cell number and mean cellular volume (10 %)[2]	Colorimetric endpoint (7%)[2]

4.2 Factors affecting the measurement of target analytes

In this part limitations and interferences on the analytes will be discussed. In general, it is always important to stick to the data of the manuals regarding sample collection, as for example, over-dilution might alter the results [11].

4.2.1 Sodium and potassium

As the biochemistry analyzer uses the same measurement method as the EPOC analyzer for most analytes, the same interferences can be expected.

Sodium

As the measurement of Na+ with the EPOC analyzer is an indirect method, a decrease (increase) of total protein will lead to an increase (decrease) of Na+ by 1.3 g/dL compared to a direct method. A high concentration of lipids doesn't affect the measurement, but Intralipid was shown to be remarkable after being tested up to 5% (lipid vol)/(plasma vol). The following substances tend to have a medically significant influence on the measurement: using evacuated collection tubes with Na+ heparin may lead to falsely higher results; a decrease of Na+ by 3 mmol/L occurs due to 20mmol/L beta-hydroxybutyrate; 16mmol/L bromide leads to an increase by 5mmol/L; contamination of samples with benzalkonium

salts cause a remarkable increase of sodium. In the case of hemodilution over 20% of plasma after using saline, Ringer, or 10% Dextrose, systematic errors can be seen when checking the results as the listed solutions are not in accordance with the ionic features of plasma. To prevent such problems, intravenous solutions that are consistent with the ionic characteristics of plasma should be used for example lactated Ringers [11]. Some examples of exogenous interferences (as 447 mg/dL ethanol, 2,2 mmol/L ibuprofen, 2 mmol/L ammonium, 0,16 mmol/L ampicillin, etc.) and endogenous interferences (as 0,26mmol/L NaCl, 10-120 mmHg pCO_2, etc.) are listed in the manual but were proven to be medically insignificant. The same applies to potassium [11].

Potassium

An increase in potassium can be seen in the case of sample hemolysis and contamination of samples with benzalkonium salts [11].

4.2.2 Glucose

As for the EPOC, the following major considerations apply: when measuring glucose using lithium or sodium heparinized tubes it should be done immediately or no anticoagulant should be used. It is not advisable to use sodium fluoride or potassium oxalate to preserve the sample. Some substances will significantly decrease glucose when being higher than the given amount: 0,47 mg/dL potassium iodide; 224 mg/dL sodium bromide; 8,2 mg/dL N-acetylcysteine; 11,8 mg/dL uric acid; 36 mg/dL mannose and 45 mg/dL xylose [11].

Using the hexokinase method, hemolysis can interfere with the procedure as more than 0.5 g of hemoglobin in the sample would make it unusable for the analysis. An erroneous increase in plasma glucose could be seen due to hypertriglyceridemia and hyperbilirubinemia [24].

For the reference method hemoglobin, bilirubin, and lipids do not interfere up to concentrations of 500 mg/dL, 40 mg/dL, and 700 mg/dL respectively. However, in some cases where IgM is increased in blood (Waldenströms macroglobulinemia), erroneous results may be seen [23].

4.2.3 Creatinine

As for the EPOC, several interferences were tested. Of note, ethylene glycol and its metabolites were tested for interference. High concentrations of glycolic acid may decrease creatinine results in the EPOC system. Bilirubin does not interfere with the measurement in

clinically important concentrations. Various exogenous (medications) and endogenous (metabolites) interferents were also tested but found insignificant [11]. The first time it was reported in 1937 and its specificity is better (than that of the Jaffe method) due to the selectivity of compounds. Nevertheless, also the enzymatic method can show interferences [21].

The reference method in this case is the so-called Jaffe method. Even though the Jaffe method is quite uncomplicated and rather cheap, its specificity is not that high as the alkaline picrate shows a reaction with other molecules having a methylene group. Some compounds such as acetone, ketones, proteins, and glucose result in incorrectly higher creatinine values, while bilirubin, lipids, and acetoacetate cause it to be lower [20, 21, 25]. In general, those interactions differ individually, but are not as common in patients already in kidney failure, seeing the real amount of creatinine rise. However in fit dogs without any disease, the plasma creatinine can be overvalued by up to 45 % [26].

To lessen such interferences, the Jaffe technique needs to undergo some changes. Often kinetic correction is used, where different phases of the reaction are checked to estimate the rate of absorbance alterations. Nonetheless, the compensations could not fully solve the problems regarding the procedures' precision [21].

The kinetic Jaffe reaction measured a lower serum creatinine concentration when adding acetoacetic acid and a higher one after adding acetone. At the highest amount that was put into the serum, the difference was around 20%. No such changes were found by the enzymatic method [20]. The addition of the maximum quantity of lipid causes a reduction of creatinine in both methods – 25% in the Jaffe method and 10% in the enzymatic one. Another decrease – 50% - can be observed by both methods with a high amount of extra bilirubin. When less bilirubin was added, the enzymatic method indicated that creatinine declined faster. In case the surplus of bilirubin is not physiological and it can be detected in urine (can be normal in male dogs) and blood, creatinine concentration drops as well (in enzymatic and Jaffe) [20]. An elevation in creatinine concentration of around 10% was measured only by the Jaffe method after the addition of glucose at a high concentration. The kinetic Jaffe technique measured a rise in creatinine of roughly 50% after adding cefazolin and 300% after cefoxitin. None of the two affected the enzymatic method. Ceftiofur showed an effect in both techniques, leading to a 5% increase and decline – the latter when applying

the enzymatic method. Neither of the two measurement reactions caused any changes after adding beta-carotene or hemoglobin [20].

4.2.4 Hemoglobin and hematocrit

For hematocrit measured with EPOC, it is recommended to carry out the analysis right away to get accurate results as the sample needs to be well mixed. An indirect proportion can be seen in the case of total protein as an increase (decrease) of 1g/dL of total protein will lead to an increase (decrease) of hematocrit by roughly 1% packed cell volume (PCV). Higher hematocrit values result from high amounts of lipids and an abnormal rise in white blood cell count. As hemoglobin is calculated from the hematocrit in the case of EPOC the results are as precise or accurate as the hematocrit measurement has been [11].

Several factors influence hemoglobin and hematocrit measurement in the ADVIA 2120i system. Unlike on the EPOC, here hemoglobin is measured whereas hematocrit is a calculated value. An important source of error may be the MCV measurement which is used to assess hematocrit [18]. Apart from the well-known interferents (e.g. lipemia artefactually increasing hemoglobin measurement), a clinically relevant interference for the present situation may have been highlighted in a study performed to find hemodialysis-induced changes in hematocrit, hemoglobin, and total protein, which found out that during dialysis hemoglobin increased more than the hematocrit. The increase after analyzing 30 hemodialysis sessions was on average 9,1 ± 7,0% (mean ± SD) for hematocrit and 10,6 ± 6,3% for hemoglobin. At the beginning and the end of each session, the arterial blood sample was taken from the arterial hemodialysis line and was analyzed using the hematology analyzer Advia 2120 [18].

5. Material and Methods

5.1 Measurement

Measurements on the EPOC system were carried out according to the manufacturer's recommendations, using syringes containing calcium-equilibrated lithium-heparin. Measurements in the Clinical Laboratory were carried out using EDTA samples on the hematology analyzer (ADvia 2120i, Siemens Healthineers, Erlangen, Germany) and serum on the clinical chemistry analyzer (Olympus AU400, Olympus, Tokyo, Japan) according to the SOP of the laboratory.

5.2 Data extraction

EPOC measurement data was extracted from the practice management system (Doki for Vets version 10.0.1786, Alpha-vet Kft, Székesfehérvér, Hungary) of the Veterinary Teaching Hospital of the University of Veterinary Medicine, Budapest.

Measurement data for the hematology and clinical chemistry parameters were extracted from the Laboratory Information and Management System (LabSoft LIMS version 2.8, NetCare.hu Kft, Kalocsa, Hungary) of the Clinical Laboratory of the Department of Clinical Pathology and Oncology.

Both of these systems use time stamps. Data for comparison was only considered if time stamps from the two systems (Doki for Vets, LabSoft LIMS) were within 1 hour of each other. This limit was created to avoid treatment or condition-related changes in the analyzed samples.

Analytes chosen for comparison were selected based on the availability of sufficient data. Chloride was omitted due to the low amount of reference data. Urea was omitted due to the lack of EPOC data.

5.3 Statistical analysis

Statistical analysis was performed using R (R version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria). Bland-Altman plots were generated along with Passing-Bablok regression analysis. Slope, intercept, and Pearson's r were generated for each analyte. Confidence intervals were generated using the bootstrap method. Precision was assessed as mean difference along with limits of agreement (95% CI). Significance was set at $p<0,05$.

The clinical laboratory's TEa quality goals were selected as acceptability criteria. These were obtained from previously published and widely accepted resources such as Westgard's Clinical Laboratory Improvement Amendments (CLIA), as presented in Table 2.

Reference interval concordance was defined as whether the EPOC results fall into the same range (below/within/above) as the results obtained from the reference method. At least 80 % of the obtained results should fall into the target range defined as the result from the reference method +/- acceptability range according to CLIA recommendations [27].

Table 2: : Acceptability criteria and sources adapted from [8, 27]

	Potassium	Sodium	Glucose	Creatinine	Hematocrit	Hemoglobin
Acceptability	+/- 0,5 mmol/L	+/- 4 mmol/L	10 %	15 %	10 %	7 %
Source	Westgard CLIA	Westgard CLIA	CLIA	CLIA	ASVCP	Westgard CLIA

6. Results

Altogether 125 individual measurements from 102 patients were obtained, from samples collected between February 2021 and August 2023. Results for comparison of K+, Na+, G, Cre, Ht, and Hb from 43, 41, 51, 107, 112, and 40 parallel measurements respectively. A summary of the results is given in Table 3. Bland-Altman and Passing-Bablok regression plots for the analytes are demonstrated in Figures 2-13.

Table 3: Results. 95 % CI for mean bias is the lower and upper limit of agreement (LOA) respectively

Note: concordance based on RI (reference interval) meaning the percentage of results that were classified similarly (i.e. within, below or above the RI); concordance meaning what percentage of the results fall into the predefined acceptability criteria

Analyte	Unit	Reference method	N	Concordance based on RI	Concordance	Concentration range (EPOC)	Slope (95 % CI)	Intercept (95 % CI)	Mean bias (95 % CI)	Correlation (r)
K	mmol/L	Olympus AU400	43	95,3 %	90,1 %	2,8 - 6,7	0,81 (0,66– 1,00)	0,41 (-0,40 – 1,66)	0,42 (0,27– 0,57)	0,85
Na	mmol/L	Olympus AU400	41	85,7 %	78,6 %	116 - 158	0,95 (0,51– 1,38)	9,61 (-52,1 – 74,1)	-1,93 (-3,74 – -0,93)	0,71
G	mmol/L	Olympus AU400	51	78,8 %	73,1 %	1,9 - 38	0,87 (0,74– 1,07)	1,34 (0,21 – 2,18)	-0,41 (-1,01 - -0,18)	0,98
Cre	µmol/L	Olympus AU400	107	87,9 %	59,3 %	32 - 1325	1,12 (1,03– 1,20)	-37,5 (-49,4 –27,5)	10,0 (-2,02 – 22,1)	0,98
Ht	%	Advia 2120i	112	76,1 %	94,7 %	19 - 74	1,07 (1,0 – 1,15)	3,53 (-6,74 – -1,0)	0 (-0,8 – 0,8)	0,94
Hb	g/L	Advia 2120i	41	73,1 %	85,4 %	79-244	1,10 (0,95– 1,25)	-12,6 (-35,6 – 6,30)	-1 (-5,70 – 3,70)	0,92

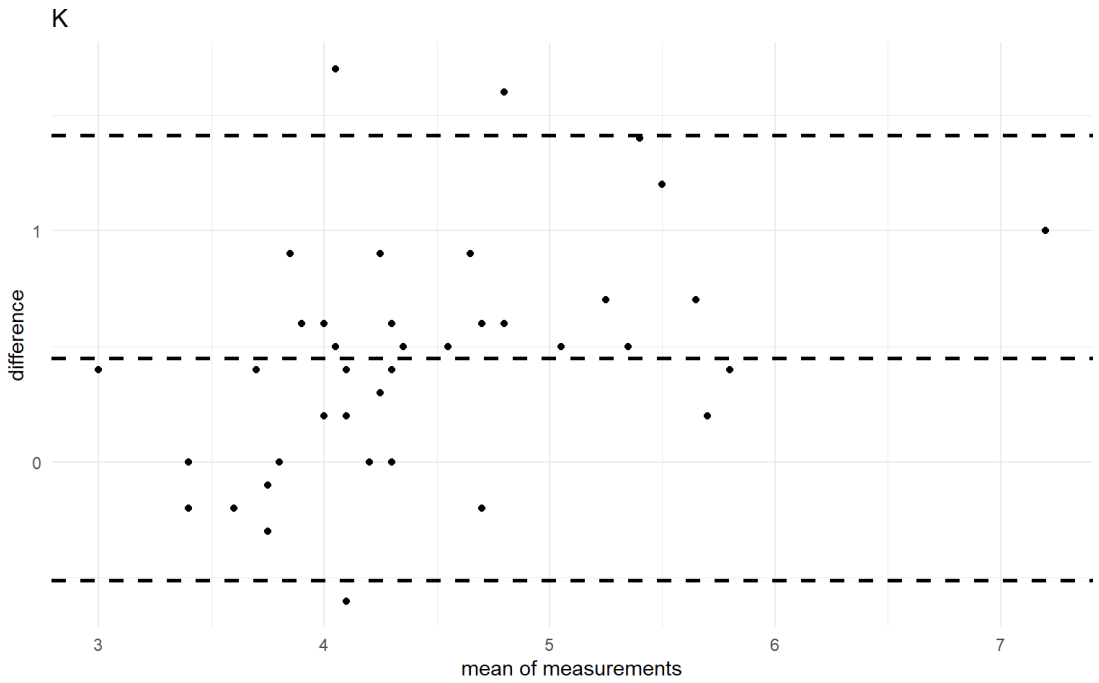

Figure 2: Bland-Altmann plot, potassium

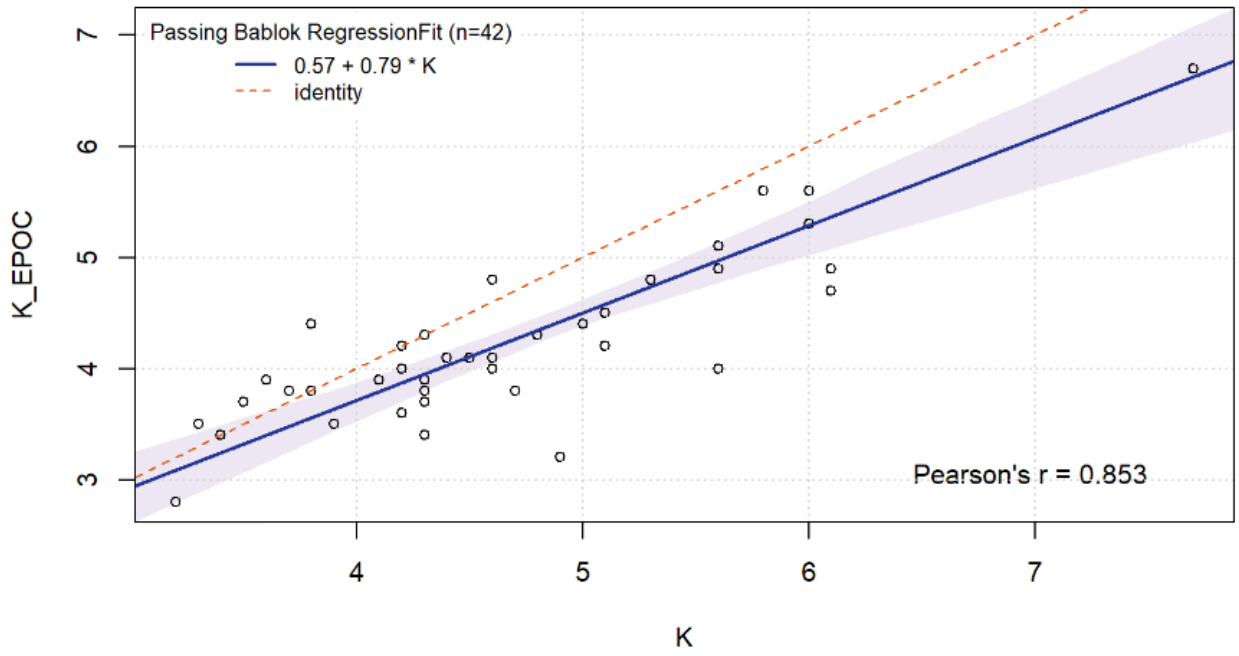

Figure 3: Passing-Bablok regression, potassium

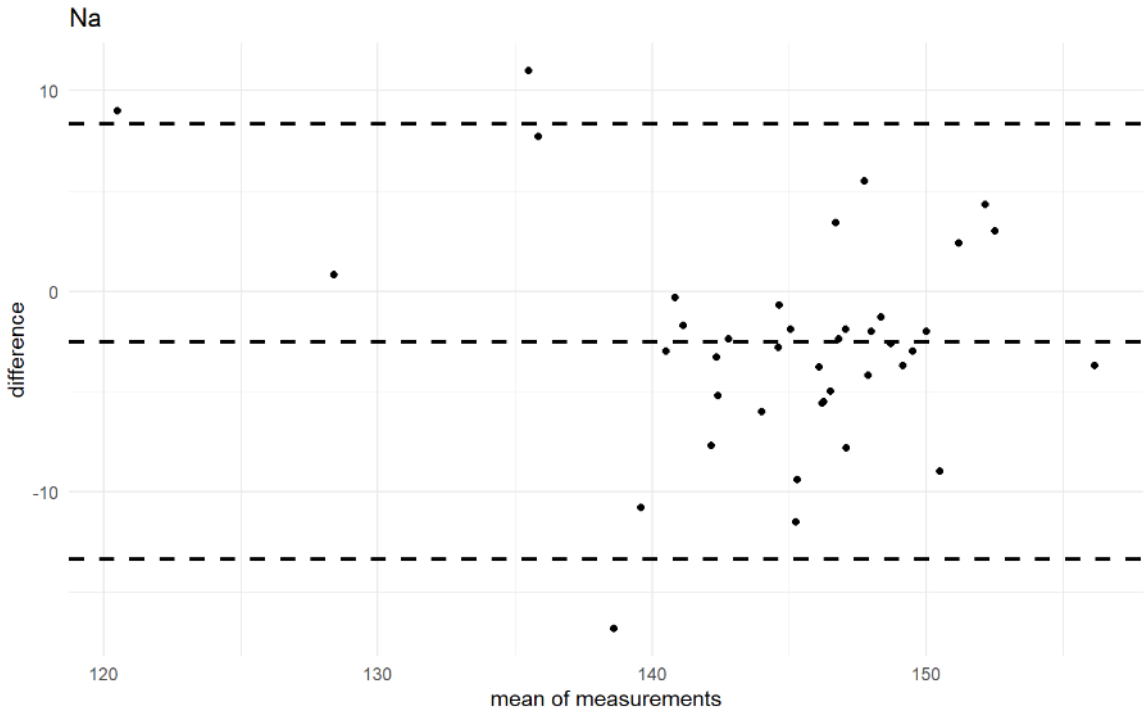

Figure 4: Bland-Altmann plot, sodium

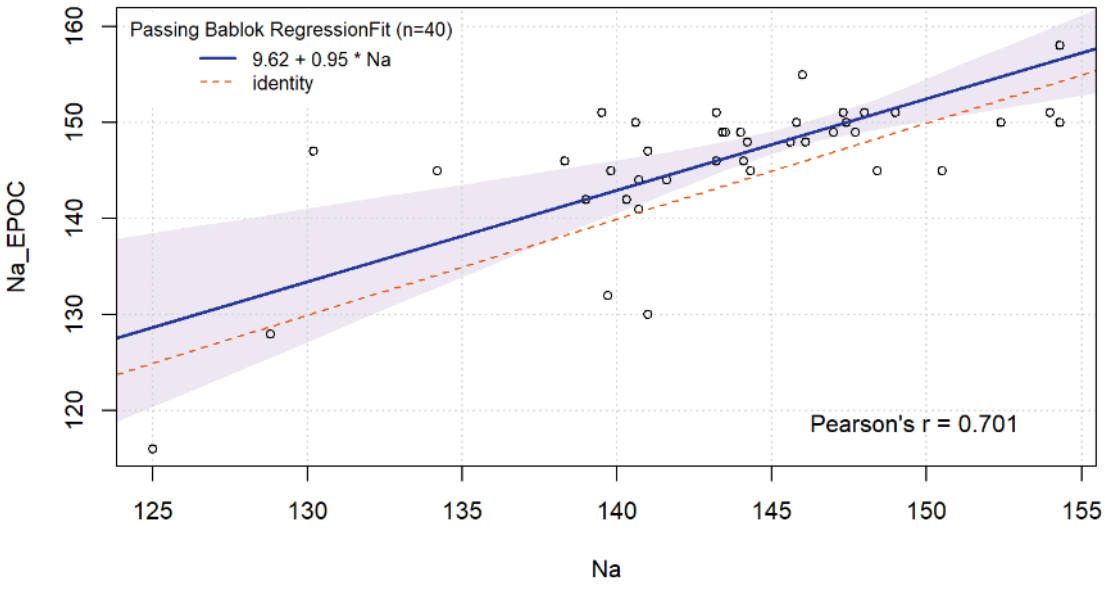

Figure 5: Passing-Bablok regression, sodium

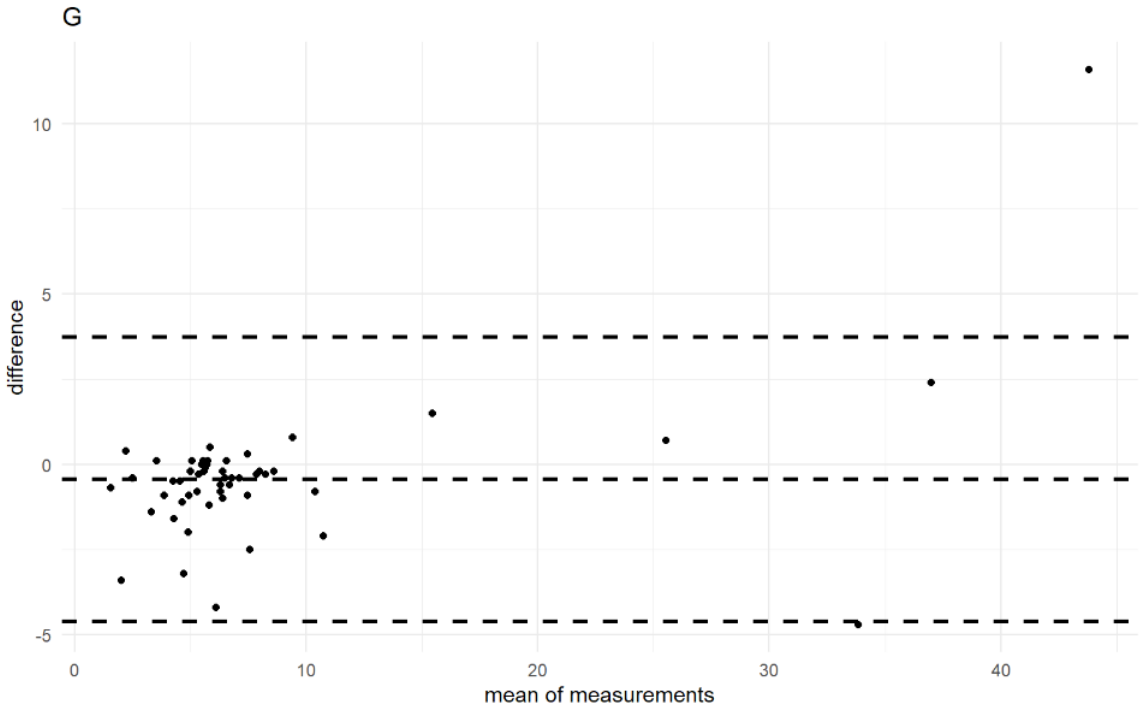

Figure 6: Bland-Altman plot, glucose

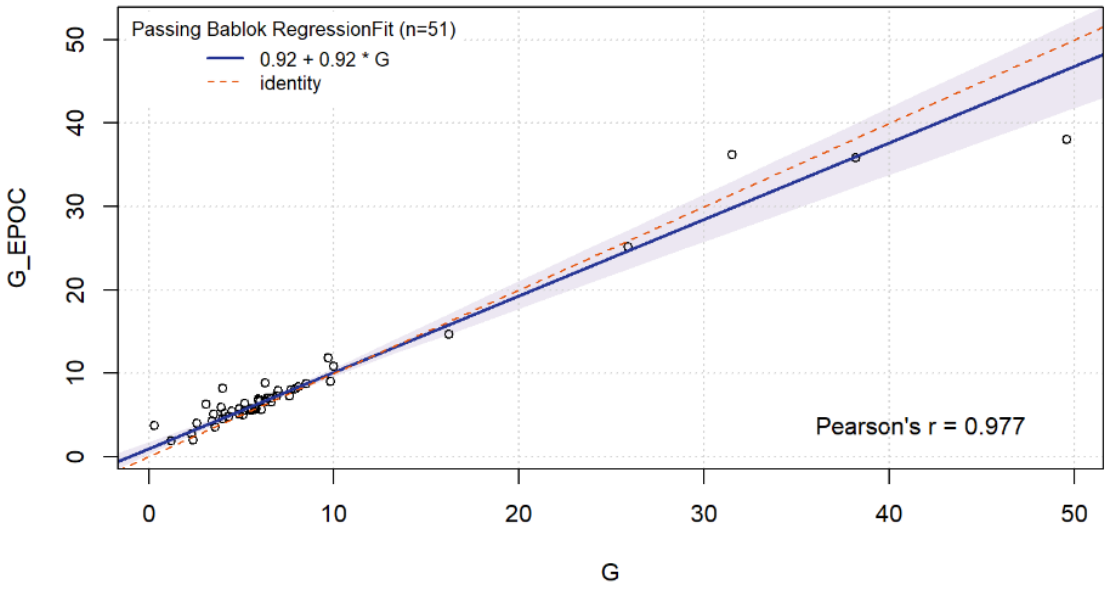

Figure 7: Passing-Bablok regression, glucose

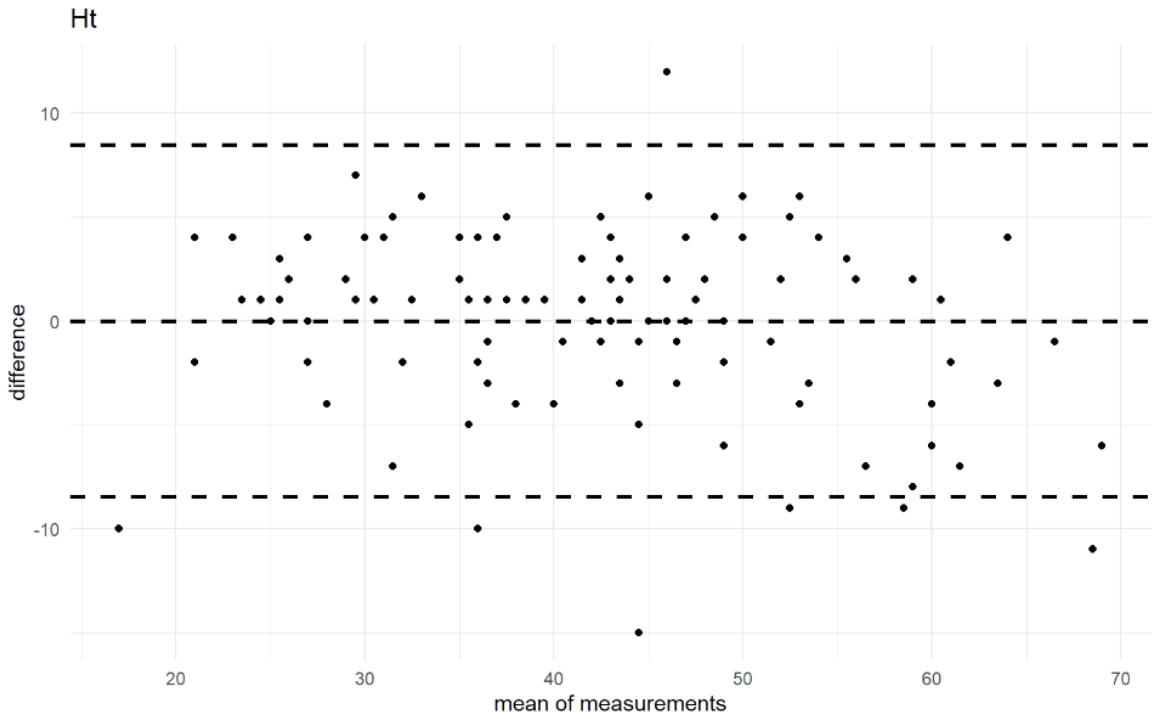

Figure 8: Bland-Altman plot, hematocrit

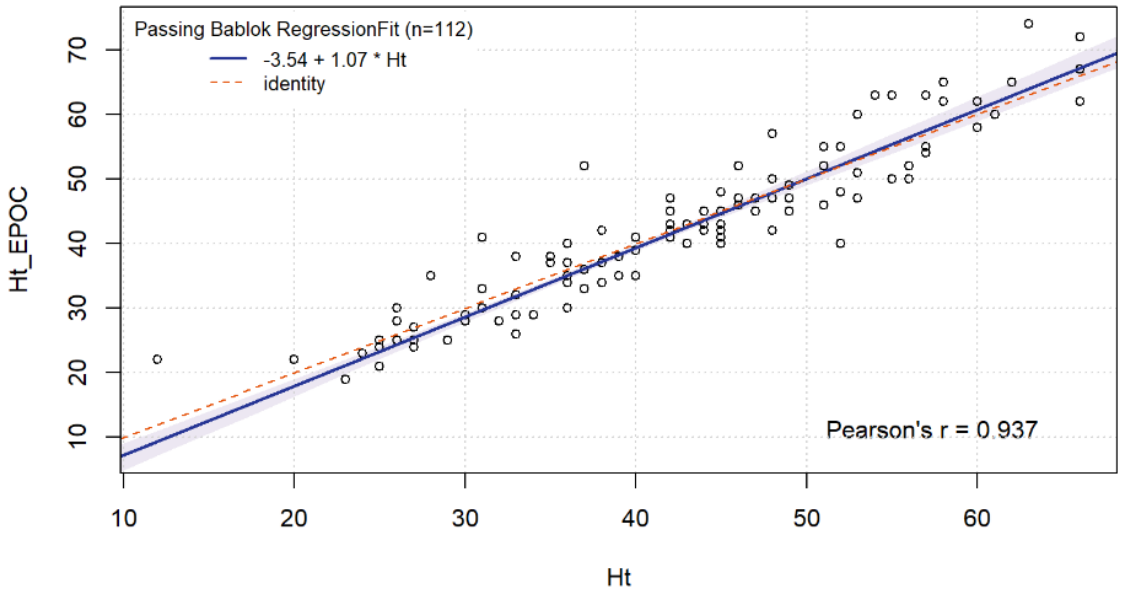

Figure 9: Passing-Bablok regression, hematocrit

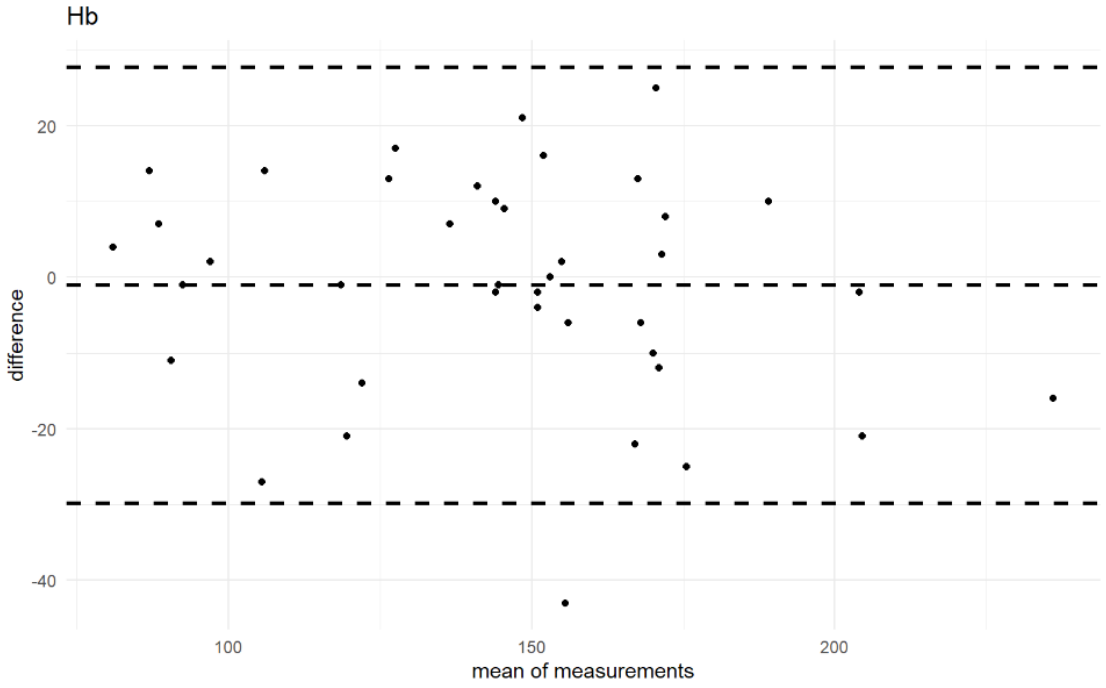

Figure 10: Bland-Altman plot, hemoglobin

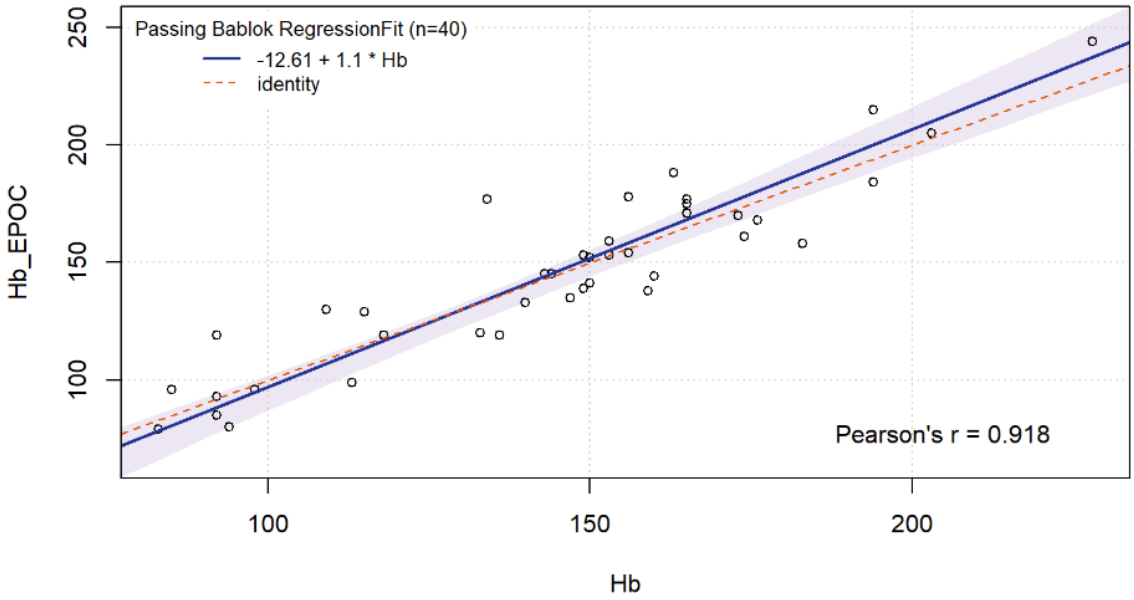

Figure 11: Passing-Bablok regression, hemoglobin

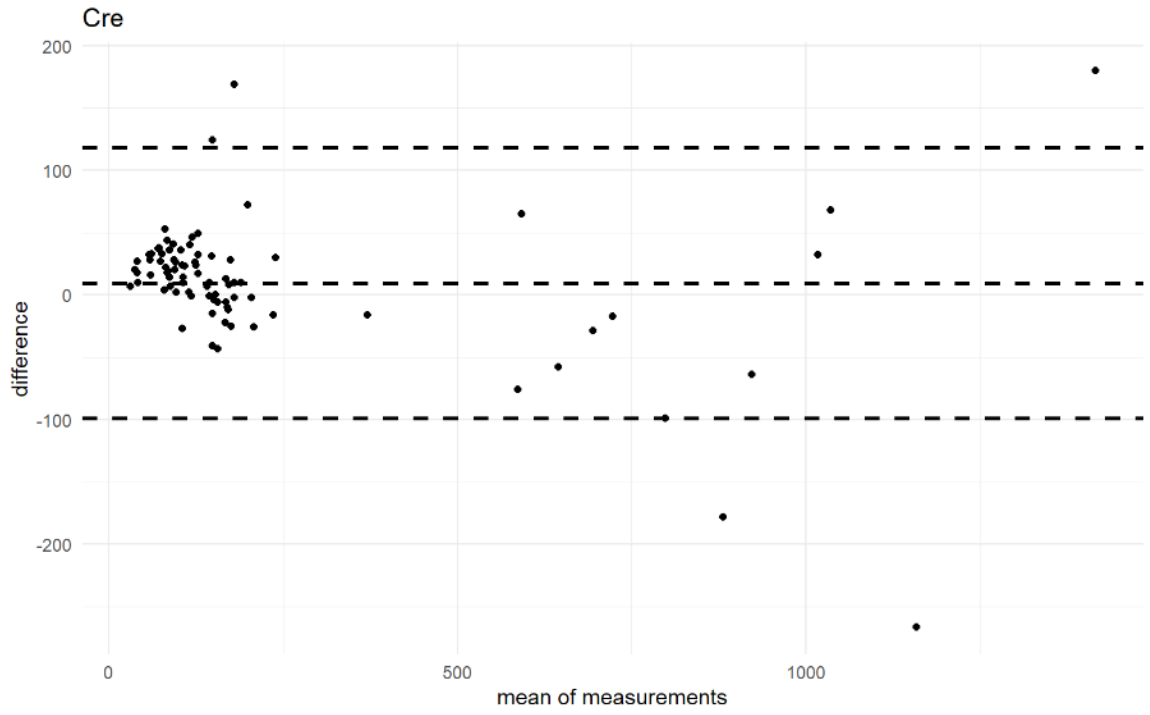

Figure 12: Bland-Altman plot, creatinine

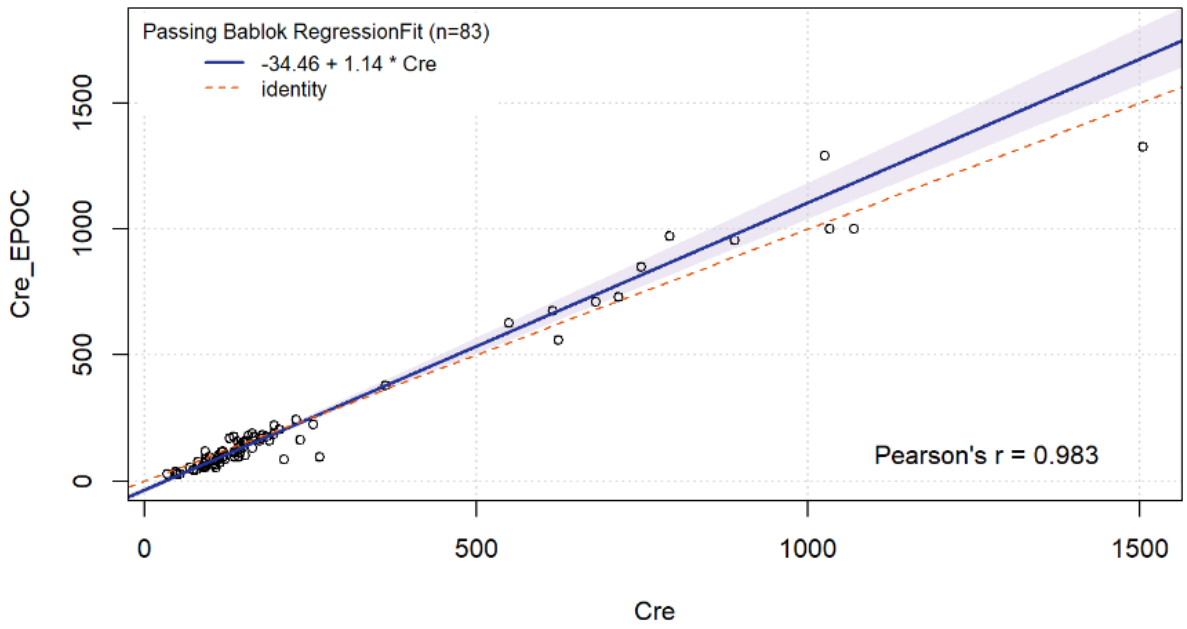

Figure 13: Passing-Bablok regression, creatinine

7. Discussion

In this study select parameters of the EPOC analyzer were assessed in clinically meaningful ranges. Instruments of the clinical laboratory were used as comparator methods.

7.1. Potassium and Sodium

Both potassium and sodium appeared to show a poor correlation in this study. 90,1 % of the obtained results for potassium and 78,6 % of those for sodium fell within the acceptability criteria. Reference interval concordance was high, which may be attributed to the relatively small sample size with a small proportion of samples falling out of the reference range for each method. However, considering the data collection method this actually may reflect real-life circumstances.

Small, but statistically significant differences (+0,27 mmol/L) were observed previously between whole blood (such as samples measured by the EPOC) and plasma (close to the serum measured by the clinical chemistry analyzer) potassium concentration in dogs. The same effect may contribute to the observed bias here [28].

A significant contribution to the observed bias may be due to the use of different ion-selective electrode methods on the two analyzers. The accuracy of the indirect ISE relies on the assumption that serum contains a predefined amount of solids (protein and lipids) and changes in the concentration of these may lead to altered results. More precisely with low TP ranges a positive bias was observed in human samples (0,64 mmol/L increase for every 10 g/L decrease of protein) [29, 30]. Potassium, although to a lesser extent, shows similar alterations [31]. These effects should be supposed for canine samples as well.

7.2. Glucose

Glucose appeared to show an excellent correlation. Still, only 73,1 % of the results fell within the acceptability criteria. Reference interval concordance was fair.

Imprecision affecting these methods is increasing both on the EPOC and the reference method with increasing glucose concentration in a similar fashion [11,23] and therefore probably does not contribute to the observed differences.

7.3. Creatinine

The correlation of creatinine to the reference method appears to be acceptable, although some differences were observed. Only 59,3 % of the results fell within the acceptability criteria. RI concordance was high.

In the case of relatively low creatinine values, the EPOC underestimated creatinine, whereas in high ranges an overestimation was observed compared to the reference method. This observation has partially been described by Márk Kiss in his thesis [1]. In short, creatinine was underestimated by the EPOC analyzer within the reference range by about 30 %.

Considering that the EPOC analyzer uses the enzymatic method whereas the reference method used here utilizes the Jaffe method, Kiss's results may be explained by the appearance of non-creatinine chromogens: substrates reacting with picric acid causing a color change and changing the result of the Jaffe reaction, causing a positive alteration which may reach up to 45 % in apparently healthy dogs. However, this unpredictable alteration should theoretically be abolished in the higher ranges of creatinine measurement and results obtained by the Jaffe method should be closer to those obtained by enzymatic methods, such as the one used in the EPOC. The EPOC appears to overestimate creatinine in the higher concentration ranges which are observed under pathologic conditions.

The International Renal Interest Society's guidelines are frequently used to grade patients suffering from either chronic kidney disease or acute kidney injury. Creatinine, as a biomarker of decreased glomerular filtration rate, is one of the major discriminators of grading, with known clinical cutoff recommendations [32]. Treatment choices are often based on these cutoff values (Table 4).

Table 4: : IRIS CKD and AKI grading based on creatinine concentration ranges adapted from [32]

CKD grade	Creatinine (µmol/L)	AKI grade	Creatinine (µmol/L)
I	<125	I	<140
II	125 – 250	II	141 – 220
III	251 – 440	III	221 – 439
IV	>440	IV	440 – 880
		V	>880

Comparing CKD or AKI IRIS grades of the results obtained from the EPOC and the reference analyzer it is apparent that some patients will be differently classified (Tables 5 and 6), potentially affecting the treatment of these individuals.

Table 5: IRIS CKD classification differences based on creatinine

IRIS CKD grade	EPOC	reference method
I	67	56
II	18	28
III	5	8
IV	17	15

Table 6:IRIS AKI classification based on creatinine

IRIS AKI grade	EPOC	reference method
I	72	61
II	13	23
III	6	7
IV	9	10
V	7	6

7.4. Hematocrit and Hemoglobin

Hematocrit and hemoglobin showed a high correlation with the reference method and moderate reference interval concordance. Results falling within the acceptance range were 94,7 and 85,4 % respectively.

Hematocrit appeared to show high similarity in terms of the classification of patients.

Most of the hemoglobin differences were seen at higher ranges meaning that most anemic patients were classified properly whereas hemoglobin concentration above the reference range was more frequently identified by the EPOC method.

7.5. General conclusions

Blood gas and acid-base measurement provides valuable information for the management of critically ill patients. Additional measured data further aids the treatment. These are frequently used for treatment decisions. The EPOC analyzer offers the possibility to obtain these results at the side of the patient. Results are provided rapidly. However, methods used in reference laboratories may be different and yield different results.

Decision limits are used in a clinical setting to aid diagnosis and treatment of diseases. The lower and upper ends of the reference intervals can be viewed as decision limits as well, especially in the cases of physiologically tightly regulated analytes such as ions and glucose;

the same is true for hemoglobin and hematocrit. As such the use of different reference intervals for the different methods may be used to compare whether diagnostic or treatment decisions would be different if the EPOC method is used instead of the reference method. It appears that reference interval concordance is moderate in this case.

Previous studies on the performance of the EPOC analyzer found performs poorly in dogs in the case of Na+, Hb, and Ht if compared with another acid-base analyzer [33]. A human study found that agreement between acid-base and standard clinical chemistry analyzers was poor for sodium and potassium [34]. Furthermore, various other differences were observed when an acid-base analyzer was tested against reference laboratory methods, similar to our study. Sodium and hemoglobin were found to perform poorly [35].

The differences may be stemming from the different measurement methods. Although ideally measuring the same analyte from the same sample should yield identical results on all devices this is not the case in real-world applications. However, this does not mean that the method itself is useless. Manufacturers test their devices extensively to assess imprecision and bias and this is demonstrated in the case of the EPOC analyzer. The manual (albeit for the same device intended for human use) is very detailed and imprecision assessment is described for each analyte in different concentration ranges, even with different sampling sites and different institutions. The observed differences rather imply that optimal treatment decisions should be based on instrument-dependent decision limits. It is also noteworthy to mention that minute changes need to be followed on a single instrument and the results from the different devices cannot be used interchangeably in this latter scenario [36].

The retrospective nature of this study did not allow for the collection of precision data with repeat measurements. Similarly, outlier testing with repeat measurements was impossible. Additionally, there is an inherent loss of control of the samples and measurements. This is balanced by the quality control measures done on the EPOC analyzer (built in each test card) and in the clinical laboratory (multilevel control measurements performed daily).

Optimally time delays between sample analyses should be restricted to a minimum. In this study, this delay was set to 1 hour which allowed the inclusion of enough patients. However, time delays between the sampling for the different measurements cannot be excluded completely.

It is desirable to perform a prospective analysis of this comparison to avoid the limitations outlined above.

8. Abstract

Aim: in a previous thesis aiming to determine reference intervals for canine venous blood significant bias was found for creatinine measurement compared with data obtained from a clinical chemistry analyzer. We intended to expand this study and investigate frequently measured parameters, namely sodium (Na+), potassium (K+), hemoglobin (Hb), hematocrit (Ht), glucose (G), and creatinine (Cre) over clinically meaningful ranges.

Materials and methods: canine venous blood samples obtained at the Teaching Hospital of the University of Veterinary Medicine, Budapest were analyzed with the EPOC system and the Advia 2120i as well as the Olympus AU400 analyzer at the clinical laboratory. Statistical methods were used to determine concordance.

Results: A good correlation was established in the case of Ht, Hb, G, and Cre whereas a significant difference was observed in the case of Na+ and K. However, proportionally higher differences were observed in the case of Hb and Cre in the higher concentration ranges. Occasionally major, seemingly random differences were observed in the case of Cre. TEa based acceptability was above 90 % in the case of K and Ht, between 80-90 % in the case of Hb, between 70-80 % in the case of Na and G, and was below 70 % in the case of creatinine.

Conclusions: The observed differences may lead to altered patient management and therefore careful interpretation of the results is suggested. A prospective study is warranted.

9. Absztrakt

Cél: egy korábbi szakdolgozatban az EPOC rendszer referenciaintervallumának felállítása során a kreatinin koncentráció szignifikáns eltérése mutatkozott a nyílt kémiai automatán végzett mérésekhez képest. Jelen dolgozatban több paraméter széles, klinikai szempontból jelentős tartományában került sor összehasonlító vizsgálatra. A vizsgált analitok a következők voltak: nátrium ion (Na+), kálium ion (K+), hemoglobin (Hb), hematokrit (Ht), glükóz (G), kreatinin (Cre).

Anyag és módszer: az Állatorvostudományi Egyetem Központi Oktató Kórházában kezelt kutyák vérmintáiból nyert eredmények vizsgálatára került sor. Az EPOC rendszer eredményei a Kórélettani és Onkológiai Tanszék Laboratóriumának Advia 2120i és Olympus AU400 automatán mért eredményeivel kerültek összevetésre, statisztikai módszerek használatával.

Eredmények: jó egyezőség mutatkozott a Ht, Hb, G és Cre esetében. Kimutatható szignifikáns eltérés észlelhető ugyanakkor a Na+ és K esetében. E mellett azonban (különösen a Hb és Cre esetében) a magas koncentrációtartományban magasabb szórás mutatkozott. A Cre egyes mintáknál kiugró eltérések mutatkoztak

Konklúzió: a fenti eltérések egyes esetekben a betegek menedzsmentját befolyásolhatják. Ennek megfelelően a leletek gondos értelmezése szükséges a klinikai döntéshozatal során. Prospektív vizsgálat elvégzése javasolható.

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Thesis progress report for veterinary students

Name of student: Thurnher Maya Georgia.....

Neptun code of the student: NOG9WC.....

Name and title of the supervisor: Márton Márialigeti DVM, department veterinarian.....

Department: Department fo Clinical Pathology and Oncology.....

Thesis title: The EPOC Blood Analysis System: Performance Analysis of Various Analytes, a Retrospective Pilot Study.....

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	02	7	Literature review	
2.	2023	03	7	Literature review	
3.	2023	04	4	Data collection	
4.	2023	05	2	Data collection	
5.	2023	06	6	Data collection	

Grade achieved at the end of the first semester: 5 (excellent)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	08	22	Statistical analysis	
2.	2023	09	5	Consultation	
3.	2023	10	3	Consultation	
4.	2023	10	17	Consultation	
5.	2023	10	31	Consultation	

Grade achieved at the end of the second semester: 5 (excellent)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: ..M. Thurner.....

Signature of the secretary of the department: .....

Date of handing the thesis in..... .....