Artykuł przeglądowy *Review*

Methods for the validation and verification of the diagnostic performance of portable electronic point-of-care analyzers for the diagnosis of subclinical metabolic diseases in dairy cows

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Received 29.07.2025 *Accepted 05.01.2026*

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Summary

Studies on the validation and diagnostic accuracy of portable electronic point-of-care analyzers (POCAs) are now widespread. These are easy-to-use, portable devices that can provide reliable and rapid on-site diagnosis in humans and animals once their accuracy has been proven. Various POCAs have been tested for analyzing the concentrations of beta-hydroxybutyrate (BHB), calcium (Ca), and glucose in blood to diagnose subclinical ketosis and hypocalcemia, which are metabolic diseases in dairy cows that significantly impact the agricultural economy. The biological, external, and qualitative factors that affect the validation and diagnostic accuracy of POCAs include the anatomical site of blood sampling, the use of anticoagulants, the type of blood sample, measurement conditions (such as environmental and human factors), the precision of the analyzers, as well as statistical analysis and its interpretation. Studies on the diagnostic accuracy and validation of POCAs require appropriate methods and statistical analyses of data. POCAs can be compared with the gold standard reference method in terms of agreement and diagnostic performance based on certain key parameters, such as mean bias, limits of bias, total error, coefficient of variation between tests, systematic constant and proportional error, area under the curve, specificity, sensitivity, positive predictive value, and negative predictive value. However, some inconsistencies and variations have been noted, as data reporting and interpretation differ from one study to another. This critical narrative review article provides an insight into the methods used to validate and verify the diagnostic accuracy of portable electronic POCAs by considering factors that influence the results of the analyses, such as external, biological, methodological, and statistical factors. It also summarized the results of studies on the validation and diagnostic accuracy of POCAs by analyzing BHB, Ca, and glucose in the blood of dairy cows for the diagnosis of subclinical ketosis and hypocalcemia.

Keywords: point-of-care analyzers, subclinical ketosis, subclinical hypocalcemia, validation, diagnostic accuracy

In recent years, several electronic point-of-care analyzers (POCAs) have been tested for their suitability for the rapid diagnosis of various diseases, such as ketosis, hypocalcemia, hyperglycemia, and hypoglycemia in dairy cows (22, 47, 65, 68, 86, 97). The validation and verification of the diagnostic accuracy of POCAs with the reference method (RM) have been considered critical, as they must be known to the user to make a reliable diagnosis. Furthermore, the environmental temperature or sample temperature has a certain effect on readings of POCAs (41, 64, 65). The site of blood collection is another factor affecting the readings of POCAs (58, 59, 83). The effect of hematocrit and total protein on blood glucose concentration (BGC) in humans (14, 25) and on beta-hydroxybutyrate (BHB) and BGC in animals has been well described (46, 64, 65). It has also been shown that the different types of anticoagulants influence BGC in plasma (24, 29), which is used as RM. The precision of a POCA is an overall quality control parameter that shows how the data have been repeatable in within-run or between-run studies compared to RM (26). The negative or posi-

tive bias, systematic or random errors, and diagnostic accuracy, including sensitivity (SE), specificity (SP), area under the curve (AUC), positive and negative predictive values (PPV, NPV), and optimized thresholds, are often only partially reported in many studies. The main parameters analyzed for the validation were blood BHB, ionized calcium (iCa), total calcium (tCa), and BGC in dairy cows. BHB and BGC were used as markers for ketosis and subclinical ketosis (SCK), whereas iCa and tCa were measured to diagnose milk fever and subclinical hypocalcemia (SCH) in dairy cows. In addition to the biological and external factors mentioned above, the methodology and interpretation of statistical analysis also vary between studies. In this narrative review (34, 80), factors and methods affecting the results of POCAs, such as external, biological and methodological factors, as well as statistical analyses performed for the validation and assessment of diagnostic performance, are briefly reviewed and discussed using selected parameters, such as BHB, iCa, tCa, and BGC, for the diagnosis of SCK and SCH in dairy cows.

The factors affecting the validation and diagnostic accuracy of POCA

Use of POCA in dairy cows

Handheld, portable, and electronic POCAs have been used to analyze BHB, iCa, tCa, and BGC on farms to provide rapid results for the prevention and treatment of SCK and SCH in dairy cows (Tab. 1, 2, and 3). SCK and SCH are economically important metabolic diseases, occurring in 50% and 22% of dairy cows, respectively, and are associated with increased incidence of postpartum diseases and reduced milk production that result in culling (2, 22, 55, 61, 73, 74), as shown in Figure 1. Many POCAs require only one drop of fresh whole blood, making them practical. A user-friendly and rapid on-farm POCA does not require centrifugation of the blood or accurate volume measurement and can quantify one or more metabolites simultaneously (47). A POCA that has been validated in dairy cows and proven to be accurate can replace the tedious and costly methods of wet chemical analysis in the laboratory. POCAs reduce the response time for disease management in cattle and swine by 0.5 days and offer cost savings to producers compared to a laboratory strategy (73). The wet-chemical analysis of BHB (42, 76), tCa (47, 51), and BGC (23, 47, 65) in serum or plasma is RM for studies on the validation and diagnostic performance of POCAs. However, validated POCAs with proven precision and diagnostic performance have also been used as RM for the validation of new POCAs, such as blood gas analyzers for the analysis of iCa (22, 82, 97). Several factors influence the outcome of validation and diagnostic accuracy studies. Figure 2 summarizes these various factors. They can be divided into the following categories: external, biological, analytical quality, methodology, and statistical analysis.

External factors

The temperature of the environment and sample. Most manufacturers of POCA recommend an ambient temperature of +5 to +32°C for use in the field. The temperature of blood samples had a significant effect on BHB (41) and glucose (64) compared to RM. The storage temperature of +5 to +32°C of the POCA and test strips did not affect the results (41). When analyzing iCa with blood gas analyzers, air bubbles in the syringe should be avoided to minimize the loss of CO_2 and the addition of O_2 to the blood samples. The heparinized polypropylene syringe should not be placed in/on ice water/block (0°C) until analysis (19). BGC was inconsistent under different storage

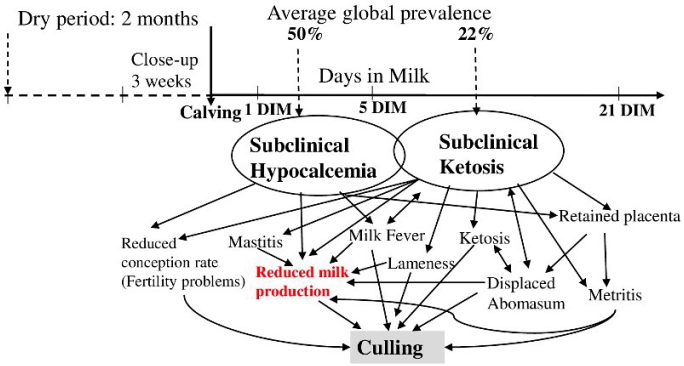


Fig. 1. Interaction of subclinical ketosis and subclinical hypocalcemia with postpartum diseases, milk production, and culling
Explanation: DIM – days in milk

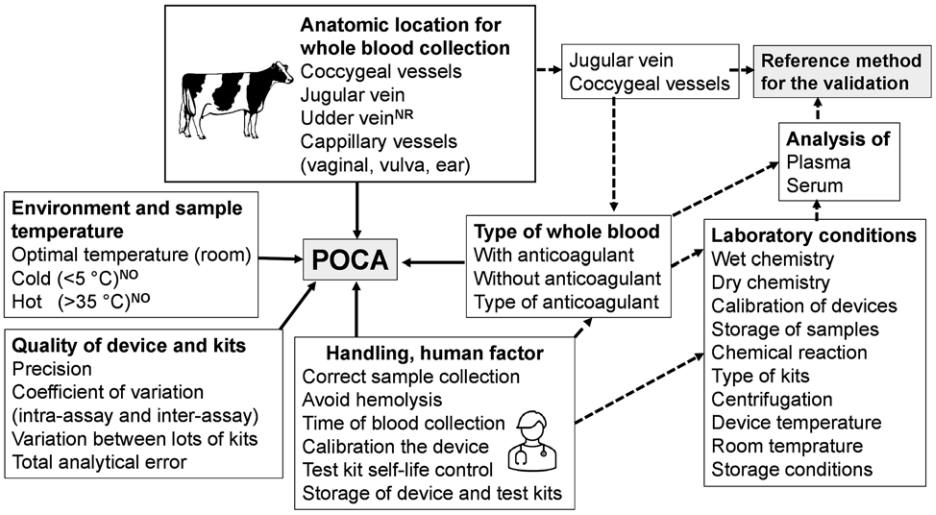


Fig. 2. Factors influencing the performance of portable electronic point-of-care analyzers (POCA) and laboratory reference methods
Explanations: Dark arrows – factors influencing the outcome of POCA in the field; dashed arrows – factors influencing the reference methods; NR – not recommended; NO – not optimal

conditions. High temperature reduced the BGC for the POC oxidase-based system (71). Indeed, POCAs can produce errors and inaccurate results at low or high ambient temperatures. This is particularly important for countries where temperatures are very high in summer and very low in winter. Monitoring and reporting the ambient temperature as an external factor during a validation study is an important part of the methodology.

The diurnal variations and human factors. Human behavior and physiology show diurnal variations (52). There were significant diurnal patterns in plasma BHB and non-esterified fatty acids when analyzing blood metabolites in dairy cows (78). A single blood sample may not accurately reflect energy status in early lactation; the timing of animal feeding should be considered (78). Blood biochemical parameters, such as glucose and some others, were affected by diurnal variations (39). People act more preventively, more slowly, and make more accurate decisions in the morning, whereas, in the evening, they make faster but less accurate decisions (52). The human contribution to laboratory errors is attributed to the complexity of tasks and human limitations, including psychophysical, organizational, workplace environment, and social factors (6). Diurnal variations and human factors may significantly influence the results of analysis.

Anticoagulants. Anticoagulation can be achieved either by binding calcium ions [(using EDTA: ethylenediaminetetraacetic acid), citrate, or fluoride] or by inhibiting thrombin (using heparin) in the blood (66). Serum is obtained from coagulated blood and is a preferred sample for analysis. However, plasma obtained with a suitable anticoagulant can be an equally acceptable sample and, in some cases, preferable to serum (66). One drop of whole blood without anticoagulants was sufficient for the analysis of BHB by POCAs, and sufficiently high SE, SP and AUC values have been reported (Tab. 1). Heparinized or EDTA blood also yielded acceptably high SE and SP values (30, 48, 89) or sufficiently high SE but low SP values (5), also in serum and EDTA plasma (72), to diagnose SCK by POCAs. The determination of BGC by POCAs in dairy cows was critical, as most results were inadequate (Tab. 3). Anticoagulants have a significant influence on the results of glucose analysis (25, 29), which also applies to RM. A combination of sodium fluoride (NaF), potassium oxalate ($K_2C_2O_4$), citrate, and EDTA to minimize glycolysis has been recommended for glucose stabilization in plasma (25, 29). In humans, the use of serum for glucose analysis is not recommended, because the glucose concentration decreases during preparation (25), and both serum and heparinized plasma with lithium showed higher glucose levels than NaF/$K_2C_2O_4$-plasma (24). The use of plasma to obtain a reliable BGC has been preferred over whole blood and serum (24, 25). However, as shown in Table 3, the serum (23, 57, 98) or plasma (47, 56, 60) of dairy cows were used for glucose analysis by RM, whereas blood samples with (heparinized or NaF) and without anticoagulant were used for analysis by POCAs. For the analysis of various parameters, including glucose, by certain POCAs, such as blood gas analyzers (23) or multiparameter POCAs (47), the use of heparinized blood is recommended. It appears that not only the type of whole blood samples (because of the influence of hematocrit), but also anticoagulants play an important role in the accurate analysis of BGC by POCAs and RM in dairy cows. The iCa concentration has been analyzed with blood gas analyzers in whole blood with lithium/sodium heparin (Tab. 2). Heparin, as the most suitable anticoagulant, was recommended for the analysis of iCa (11) in whole blood and tCa in plasma (66). BGC and tCa concentrations in serum and heparinized plasma did not differ significantly, but BGC was significantly low in citrate-plasma, whereas calcium was significantly low in EDTA-plasma (66).

Biological factors

The effect of the anatomical site of blood sampling. It is obvious that the site from which blood was taken influenced the parameters. Blood from the mammary veins (*V. epigastrica cranialis superficialis* or *V. subcutanea abdominis*) contained less BHB than blood samples from *V. jugularis* and coccygeal vessels (artery or veins), which is why blood sampling from the mammary veins is not recommended (58, 94). On the other hand, the capillary vessels of the ear contained more BHB than serum and whole blood from the coccygeal artery or veins (83). This is probably due to a difference in BHB consumption in tissues supplied by these veins, particularly in the udder tissues (94). In most studies, blood samples were taken from the coccygeal vessels (artery or vein) or *V. jugularis* of dairy cows. However, for practical reasons, the researchers also took blood samples from capillary vessels (ear and vaginal skin by puncture with lancets), and the results varied between studies (Tab. 1, 2, and 3). Therefore, the determination of the blood sampling site and the type of blood sample (whole blood, serum, plasma) is an important part of the methodology of validation studies.

The effect of hematocrit. Hematocrit has a significant effect on the BHB concentration in dairy cows, as high hematocrit leads to a high blood BHB concentration (64). Most POCAs are designed for humans, but are also used in animals. In humans, hematocrit influences BGC (14). The glucose equivalent concentrations of whole blood and plasma differ in humans because of the different dissolution of glucose in water and the water content of the cytosol of red blood cells (71%) and plasma (93%) (25). Human blood plasma with a normal hematocrit contains a 10-15% higher glucose concentration than a corresponding volume of a whole blood sample. A correction factor of 1.11 has been recommended for the conversion of whole blood glucose to plasma equivalent glucose in molar form (hematocrit: 43%). There is a negative correlation

Tab. 1. Results of validation and diagnostic accuracy studies of handheld and portable electronic point-of-care analyzers (POCA) for diagnosing subclinical ketosis by blood analysis of beta-hydroxybutyric acid (BHB) concentration in dairy cows

Ref.	POCA	RM device or kit	Blood sample for RM	Blood sample for POCA	MB of RM-POCA (mg/dl)	LA or (±) SD* of MB (mg/dl)	IA-CVM of POCA (%)	Regression (PB/Deming)	BHB-TRH for RM (mg/dl)	O-TRH for POCA (mg/dl)	SE (%)	SP (%)	AUC
2	TaiDoc	WellionVet Belua	WB-Coccygeal v.	WB-Coccygeal v.	0.30#	0.31/–0.71	NR	Const./Propor. errors	≥ 1.20	DNP	64.7	91.0	0.78
30	BoviLab	CA-90; Furuno Electric	Hepar-plasma (coccygeal v.)	Hepar-WB (coccygeal v.)	0.019	0.19/–0.15	4.5	DNP	≥ 1.20	DNP	94.7	99.5	NR
30	BoviLab	CA-90; Furuno Electric	Hepar-plasma (coccygeal v.)	Hepar-WB (coccygeal v.)	0.019	0.19/–0.15	4.5	DNP	≥ 1.00	DNP	86.2	99.0	NR
42	Freestyle Precision Neo	Ranbut RX	Serum-Coccygeal v.	WB-Coccygeal v.	–0.01	0.62/–0.61	NR	No errors	≥ 1.20	1.00	87.0	90.5	0.94
42	BHB-Check (TaiDoc)	Ranbut RX	Serum-Coccygeal v.	WB-Coccygeal v.	0.06	0.64/–0.52	NR	Const. error	≥ 1.20	0.80	91.6	82.7	0.93
42	WellionVet Belua	Ranbut RX	Serum-Coccygeal v.	WB-Coccygeal v.	–0.27	0.38/–0.92	NR	Const./Propor. errors	≥ 1.20	0.80	89.7	86.2	0.93
42	GlucoMen LX Plus	Ranbut RX	Serum-Coccygeal v.	WB-Coccygeal v.	–0.08	0.72/–0.84	NR	Const./Propor. errors	≥ 1.20	1.10	71.8	89.5	0.88
48	WellionVet Belua	Cobas 6000/c501	Hepar-plasma (Jugular)	Jugular vein (heparin)	–0.01	0.58/–0.60	NR	NR	≥ 1.20	DNP	89.0	99.0	0.99
48	WellionVet Belua	Cobas 6000/c501	Hepar-pasma (Jugular)	Vaginal (Capillary WB)	0.06	0.58/–0.40	NR	NR	≥ 1.20	DNP	96.0	98.0	1.00
76	BHBCheck	EKF Diagnostics	Serum-Coccygeal v.	WB-Coccygeal v.	–0.05	0.34/–0.44	NR	NR	≥ 1.20	DNP	89.0	84.0	0.98
76	BHBCheck	EKF Diagnostics	Plasma with C2K2O4/NaF	WB-Coccygeal v.	–	–	NR	NR	≥ 1.20	DNP	91.0	93.0	0.98
76	Precision Xtra	EKF Diagnostics	Serum-Coccygeal v.	WB-Coccygeal v.	–0.09	0.33/–0.51	NR	NR	≥ 1.20	DNP	100	84.0	0.98
76	Precision Xtra	EKF Diagnostics	Plasma with C2K2O4/NaF	WB-Coccygeal v.	–	–	NR	NR	≥ 1.20	DNP	97.0	92.0	0.99
57	FreeStyle Precision Neo	Hitachi 911 Analyzer	Serum-Coccygeal v.	WB- Coccygeal v.	DNP	DNP	1.3	NR	≥ 1.10	DNP	100	92.0	NR
57	FreeStyle Precision Neo	Hitachi 911 Analyzer	Serum-Coccygeal v.	WB-Coccygeal v.	DNP	DNP	1.3	NR	≥ 1.20	DNP	98.0	95.0	NR
98	Precision Xtra	Erba XL-200, Germany	Serum-Coccygeal v.	WB-Coccygeal v	0.07#	–0.30/0.45	0.90	No errors	≥ 1.20	DNP	94.0	94.0	NR
98	Precision Xtra	Erba XL-200, Germany	Serum-Coccygeal v.	WB-Coccygeal v	0.07#	–0.30/0.45	0.90	No errors	≥ 1.40	DNP	93.0	95.0	NR
5	Precision Xtra	Randox Laboratories	Hepar-plasma	Hepar-WB	–0.34	0.30/–0.99	0.0	NR	≥ 1.20	DNP	100	73.5	NR
5	NovaVet	Randox Laboratories	Hepar-plasma	Hepar-WB	0.08	0.55/–0.40	8.8	NR	≥ 1.20	DNP	94.9	74.4	NR
5	TaiDoc	Randox Laboratories	Hepar-plasma	Hepar-WB	–0.21	0.31/–0.73	7.7	NR	≥ 1.20	DNP	100	73.5	NR

Ref.	POCA	RM device or kit	Blood sample for RM	Blood sample for POCA	MB of RM-POCA (mg/dl)	LA or (±) SD* of MB (mg/dl)	IA-CVM of POCA (%)	Regression (PB/Deming)	BHB-TRH for RM (mg/dl)	O-TRH for POCA (mg/dl)	SE (%)	SP (%)	AUC
5	Nova Max	Randox Laboratories	Hepar-plasma	Hepar-WB	0.26	0.73/–0.21	11.5	NR	≥ 1.20	DNP	91.8	100	NR
83	FreeStyle Precision Neo	NR	Serum-Coccygeal v.	Capillary v. (left ear)	0.22	0.49*	11.7	NR	≥ 1.20	1.30	100	93.0	0.99
83	FreeStyle Precision Neo	NR	Serum-Coccygeal v.	Capillary v. (right ear)	0.23	0.51*	11.7	NR	≥ 1.20	1.30	100	94.0	0.99
83	FreeStyle Precision Neo	NR	Serum-Coccygeal v.	WB-Coccygeal v.	0.02	0.21*	11.7	NR	≥ 1.20	1.10	100	95.0	1.00
45	FreeStyle Precision	Cobas 6000/501c	Serum-Coccygeal v.	Capillary v. (vulva skin)	0.08	0.19*	7.9	Const./Propor. errors	≥ 1.20	1.00	100	76.0	96.0
45	GlucoMen LX Plus	Cobas 6000/501c	Serum-Coccygeal v.	Capillary v. (vulva skin)	–0.07	0.36*	8.3	Const./Propor. errors	≥ 1.20	1.10	80.0	89.0	87.0
45	NovaVet	Cobas 6000/501c	Serum-Coccygeal v.	Capillary v. (vulva skin)	–0.01	0.43*	10.9	No errors	≥ 1.20	1.20	89.0	84.0	90.0
45	FreeStyle Precision	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	0.02	0.12*	7.9	Const./Propor. errors	≥ 1.20	1.20	100	93.0	99.0
45	GlucoMen LX Plus	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	–0.10	0.21*	8.3	Const./Propor. errors	≥ 1.20	1.10	94.0	85.0	94.0
45	NovaVet	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	–0.06	0.17*	10.9	Const./Propor. errors	≥ 1.20	1.10	100	83.0	97.0
72	Precision Xtra	Randox Laboratories	Serum-Coccygeal v.	Serum (coccygeal v.)	–0.63	–1.45/–0.19	NR	NR	≥ 1.20	DNP	100	51.0	0.76
72	Precision Xtra	Randox Laboratories	EDTA-Plasma (coccygeal v.)	EDTA-Plasma (coccygeal v.)	–0.50	–1.00/0.00	NR	NR	≥ 1.20	DNP	100	55.0	0.78
59	NovaVet	AU480	Serum-Coccygeal v.	WB-Coccygeal v.	–0.07	0.34/–0.48	NR	NR	≥ 1.20	1.20	97.0	82.0	0.97
41	FreeStyle Precision	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	0.04#	0.15*	5.7	NR	≥ 1.20	1.20	98.0	90.0	0.99
41	FreeStyle Precision	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	0.04#	0.15*	5.7	NR	≥ 1.40	1.40	100.0	97.0	1.00
41	GlucoMen LX Plus	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	–0.12#	0.22*	0.89	NR	≥ 1.20	1.10	80.0	87.0	0.94
41	GlucoMen LX Plus	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	–0.12#	0.22*	0.89	NR	≥ 1.40	1.30	86.0	96.0	0.98
89[1]	Optium Xceed	Merck Microlab 200	EDTA-Plasma (Jugular)	Jugular WB (EDTA)	0.04	0.22/–0.29	NR	No errors	≥ 1.20	DNP	85.0	95.0	NR
89[1]	Optium Xceed	Merck Microlab 200	Plasma-EDTA (Jugular)	Jugular WB (EDTA)	0.04	0.22/–0.29	NR	No errors	≥ 1.40	DNP	90.0	98.0	NR
40[1]	Precision Xtra	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	0.048	0.26*	NR	NR	≥ 1.20	DNP	88.0	96.0	NR
40[1]	Precision Xtra	Cobas 6000/501c	Serum-Coccygeal v.	WB-Coccygeal v.	0.048	0.26*	NR	NR	≥ 1.40	DNP	96.0	97.0	NR

Explanations: Ref. – reference number; RM – reference method; IA-CVM – intra-assay coefficient of variation for medium concentration; BHB-TRH – beta-hydroxybutyric acid threshold for RM; O-TRH – optimized threshold for POCA; SE – sensitivity; SP – specificity; AUC – area under the curve; MB – mean bias of POCA determined by Bland-Altman; WB – whole blood; Coccygeal v. – coccygeal vessels; Capillary v.– capillary vessels; PB/Deming – Passing-Bablok/Deming regression equation; Const./Propor errors – constant and proportional errors; EDTA – ethylendiamintetraacetat; DNP – data not presented (statistics were performed, but parameter was not found); NR – not reported (statistics were not performed); LA – limits of agreement by Bland-Altman analysis; * – standard deviation (±); # – MB and LA refer to = POCA – RM (positive MB refers to overestimation, negative MB refers to underestimation); [1] – BHB value was converted from µM to mM.

Tab. 2. Results of validation and diagnostic accuracy studies of handheld and portable electronic point-of-care analyzers (POCA) for diagnosing subclinical hypocalcemia by analysis of ionized and total blood calcium concentrations (mmol/L) in dairy cows

Ref.	POCA	RM device or kit	Blood sample for RM	Blood sample for POCA	MB of RM-POCA (mmol/L)	LA or (±) SD* of MB (mmol/L)	IA-CVM of POCA (%)	Regression (PB/Deming)	TRH for RM (mmol/L)	O-TRH for POCA (mmol/L)	SE (%)	SP (%)	AUC
15	Horiba LAQUAtwin Ca-11C	iSTAT-1	Hepar-WB for iCa (coccygeal v.)	Hepar-WB for iCa (coccygeal v.)	0.004**	0.11/(–0.10)	NR	No errors	NR	NR	NR	NR	NR
47	POC (I.B Co., Ltd. Tokyo)	Cayman Chem., Ann	Hepar-plasma for tCa (coccygeal v.)	Hepar-WB for tCa (coccygeal v.)	0.06	0.22*	5.25	NR	NR	NR	NR	NR	NR
22	EDAN i 15 Vet	Gem Premier 3000	Hepar-WB for iCa (coccygeal v.)	Hepar-WB for iCa (coccygeal v.)	0.05**	0.17/–0.07	NR	Const. error	iCa < 1.00	iCa ≤ 1.04	93.0	94.0	0.97
22	EDAN i 15 Vet	Gem Premier 3000	Hepar-WB for iCa (coccygeal v.)	Hepar-WB for iCa (coccygeal v.)	0.05**	0.17/–0.07	NR	Const. error	iCa < 1.00	iCa ≤ 1.04	93.0	94.0	0.97
22	EDAN i 15 Vet	Gem Premier 3000	Hepar-WB for iCa (coccygeal v.)	Hepar-WB for iCa (coccygeal v.)	0.05**	0.17/–0.07	NR	Const. error	iCa < 1.00	iCa ≤ 1.04	93.0	94.0	0.97
22	EDAN i 15 Vet	Mindray BS 120 Vet	Serum for tCa (coccygeal v.)	Hepar-WB for iCa (coccygeal v.)	NA	NA	NR	NA	tCa < 2.15	NR	57.4	81.7	0.72
22	Gem Premier 3000	Mindray BS 120 Vet	Serum for tCa (coccygeal v.)	Hepar-WB for iCa (coccygeal v.)	NA	NA	NR	NA	tCa < 2.15	NR	57.4	80.3	0.75
82	Gem Premier 3500	i-Stat 1	Hepar-WB for iCa (jugular vein)	Hepar-WB for iCa (jugular vein)	0.031**	0.133/–0.071	NR	No errors	NR	NR	NR	NR	NR
82	Gem Premier 3500	iCa Checker	Hepar-WB for iCa (jugular vein)	Hepar-WB for iCa (jugular vein)	0.038**	0.194/–0.118	NR	No errors	NR	NR	NR	NR	NR
79	TD-5220 Vet Ca	Cobas Calcium Gen	Serum for tCa (coccygeal v.)	Na-Hepar-WB for tCa (coccygeal v.)	0.26**	0.8/91.41	1.30	NR	tCa ≤ 2.14	2.40	58.0	64.0	NR
79	TD-5220 Vet Ca	Cobas Calcium Gen	Serum for tCa (coccygeal v.)	Na-Hepar-plasma for tCa (coccygeal v.)	0.53**	0.13/0.92	1.30	NR	tCa ≤ 2.14	2.55	72.0	86.0	NR
97	i-Stat	Radiometer ABL800 FLEX	Hepar-WB for iCa (jugular vein)	Hepar-WB for iCa (jugular vein)	0.00	0.07/–0.07	0.7	No errors	iCa < 1.00	iCa ≤ 0.99	100.0	98.9	NR
97	i-Stat	Radiometer ABL800 FLEX	Hepar-WB for iCa (jugular vein)	Hepar-WB for iCa (jugular vein)	0.00	0.07/-0.07	0.7	No errors	iCa < 1.18	iCa ≤ 1.21	94.8	64.6	NR
68	VetScan i-STAT	ABL-800 FLEX	Radiometer ABL800 FLEX	Hepar-WB for iCa (coccygeal v.)	–0.04**	–0.01/–0.07	2.1	Const./ propor. errors	NR	NR	NR	NR	NR
68	Prototype POCA	ABL-800 FLEX	Radiometer ABL800 FLEX	Hepar-WB for iCa (coccygeal v.)	0.04**	0.18/0.01	2.1	Const./ propor. errors	NR	NR	NR	NR	NR
51	i-Stat	Beckman Coulter 640e	Serum for tCa (coccygeal v.)	WB for iCa (coccygeal v.)	NA	NA	NR	NA	tCa < 2.00	iCa < 1.00	39.0	98.0	NR
51	i-Stat	Beckman Coulter 640e	Serum for tCa (coccygeal v.)	WB for iCa (coccygeal v.)	NA	NA	NR	NA	tCa < 2.00	iCa < 1.17	94.0	84.0	0.93
51	i-Stat	Beckman Coulter 640e	Serum for tCa (coccygeal v.)	WB for iCa (coccygeal v.)	NA	NA	NR	NA	tCa < 2.125	iCa < 1.17	82.0	94.0	0.93
51	VetTest Chemistry	Beckman Coulter 640e	Serum for tCa (coccygeal v.)	Serum for tCa (coccygeal v.)	–0.22	–0.02/–0.42	0.8	NR	tCa < 2.00	tCa < 2.23	87.0	89.0	0.95
51	VetTest Chemistry	Beckman Coulter 640e	Serum for tCa (coccygeal v.)	Serum for tCa (coccygeal v.)	–0.22	–0.02/–0.43	0.8	NR	tCa < 2.125	tCa < 2.30	87.1	89.1	0.97

Explanations: Ref. – reference number; RM – reference method; IA-CVM – intra-assay coefficient of variation for medium concentration; iCa – ionized calcium; tCa – total calcium; TRH – threshold (iCa and tCa) for RM; O-TRH – optimized threshold for POCA; SD* – standard deviation; SE – sensitivity; SP – specificity; AUC – area under the curve; MB – mean bias; WB – whole blood; Hepar-WB – heparinized whole blood; PB/Demin – Passing-Bablok/Deming regression equation; NA – not applicable; NR – not reported; LA – limits of agreement by Bland-Altman analysis; Const. error – Constant error; Const./propor. errors – constant and proportional errors; ** – MB and LA refer to = POCA – RM (positive MB refers to overestimation, negative MB refers to underestimation); Remark – calcium units have been converted from mg/dl to mmol/L by multiplication with 0.02495.

between hematocrit and total BGC. If hematocrit is abnormal due to disease, total BGC should be adjusted to the normal hematocrit of 0.43 (43%) by multiplying by 0.84/(0.93 – 0.22 × hematocrit) (25). Therefore, an equation for humans has been recommended: glucose (molar, plasma) = 1.11 × glucose (molar, whole blood) by a POCA (25). POCAs use an algorithm that predicts that intra-erythrocytic (corpuscle) glucose and hematocrit levels are similar in humans and cows. However, intra-erythrocytic glucose in adult cattle was lower than in humans at 18% plasma glucose on a molar basis, and median hematocrit was also lower in adult cattle (0.33; 33%) than in humans (0.43; 43%) (65). Therefore, POCAs cannot be used accurately with bovine whole blood due to the algorithm optimized for human blood. There are 4 independent factors that influence the SE of BGC analysis in humans: hematocrit (43%), plasma protein (7 g/dL), r = 1.0 (molal intraerythrocytic glucose by POCA/molal plasma glucose by POCA), and free water content of erythrocytes (*fe* = 0.71) in the plasma glucose equation. Instead, a new equation based on a median hematocrit of 33%, total protein of 6 g/dL, r of 0.25, and *fe* of 0.65 was recommended for calculating the plasma glucose equivalent of whole blood in cattle (65): Plasma glucose = 0.66 × molar plasma glucose by POCA + 15. Blood glucose = 0.90 × molar blood glucose by POCA + 15. BGC fluctuates significantly during the transition period in dairy cows (23, 70). Overestimation and underestimation of BGC by POCAs can lead to increased SE and SP for hyperglycemia and hypoglycemia in dairy cows (Tab. 3), which is due to a higher probability of detecting hypoglycemia or hyperglycemia at high or low BGC during the transition period (23). The hematocrit-adjusted plasma glucose concentration in dairy cows was not reported in most validation studies (Tab. 3), except by Karapınar et al. (46) in calves and by Megahed et al. (65). Accurate analysis of plasma-equivalent BGC by POCAs in whole blood is very complex and uncertain in dairy cows because of the effects of hematocrit, anticoagulants, blood sample type, and collection time.

Analytical quality: Precision and total error

Precision. A POCA must be tested for intra-assay and inter-assay precision (coefficient of variation for within-run and between-run), which refers to the closeness of repeated individual measurements in the same samples, meaning the quality and reliability of the POCA and its kit. It can be tested by performing at least five analyses in the same samples at low, normal, and high concentrations (intra-assay) and at different time points (inter-assay) to determine the coefficient of variation (CV) in% (26). CV must not be higher than 15% for each concentration (26). CV can be calculated using the standard deviation and the mean value (37).

Coefficient of Variation (CV)

$$CV\ (\%) = 100 \times \frac{\text{Standard deviation}}{\text{Mean}}$$

Mean bias. The difference between the mean values of the results of two methods (Mean bias = mean value of RM – mean value of the POCA measurement) is referred to as the mean bias, which is indicated in the Bland-Altman plots of agreement with a confidence interval (CI) of 95%. The mean bias in units can be converted into a mean bias in% by the following formula (28, 38).

Calculation of Mean Bias (%)

$$\text{Mean bias (\%)} = \frac{\text{Mean (reference)} - \text{Mean (measured)}}{\text{Mean (reference)}} \times 100$$

Total analytical error (TE). It is also recommended that TE be calculated, as it is not indicated by the mean bias. TE reflects the sum of random error (imprecision) and systematic error (bias or inaccuracy or a constant and proportional error) (28, 44). TE can be calculated using the mean bias and standard deviation, which is also referred to as the total observable error (TOE) (28, 38).

Calculation of Total Errors (TE)

TE (in units) = mean bias + 2 x standard deviation

TE (in %) = mean bias (%) + 2 x coefficient of variation (%)

TOE should not exceed the total allowable error (TAE) in method comparison studies (TOE < TAE). A highly accurate and precise measurement by a new test method means a very low bias and CV or standard deviation (28, 38). TOE is specific to a particular POCA or device and can change with the concentration or activity of the parameter. It shows the type 1 error (false positive, alpha error), type 2 error (false negative, beta error), and Z-score (probability of TOE estimate) (28). TAE in the assessment of quality may vary according to animal species, disease, analyte concentration (low, normal, high), clinical use, type of laboratory, and at different thresholds for "normal" or different clinical diseases (38).

According to the International Organization for Standardization, when analyzing BGC measured by a POCA in humans, any new method must achieve 95% of BGC results within ± 15% for BGC < 100 mg/dl and > 100 mg/dl compared to the laboratory RM (43). A TAE of 10% for low concentrations, 20% for high concentrations, and within-run precision have been reported for BGC (38). According to the American Clinical Laboratory Improvement, a TAE of 6% for low BGC and 10% for high BGC was reported (38). Westgard (91) recommended 6.96% TAE and 4.6% maximum allowable uncertainty for BGC. TAE for tCa has been reported as 10% for low, normal, and high concentrations (38), but it is 8% according to limits established in Canada, University of Physicians and Surgeons of Saskatchewan (38). Westgard (91) recommended a maximum allowable uncertainty of 1.8% for tCa according to the European Federation of Clinical Chemistry and Laboratory Medicine and

Tab. 3. Results of validation and diagnostic accuracy studies of handheld and portable electronic point-of-care analyzers (POCA) for diagnosing hypoglycemia and hyperglycemia by analyzing blood glucose concentration (BGC) in dairy cows

Ref.	POCA	RM device or kit	Blood sample for RM	Blood sample for POCA	MB of RM-POCA (mg/dl)	LA or (±) SD* of MB (mg/dl)	IA-CVM of POCA (%)	Regression (PB/Deming)	BGC-TRH for RM (mg/dl)	O-TRH for POCA (mg/dl)	SE (%)	SP (%)	AUC
23	Edan i15Vet	Mindray BS120	Serum (coccygeal v.)	Hepar-WB (coccygeal v.)	–10.90#	12.4/–34.1	NR	No error	< 40.0	NR	71.4	70.6	0.71
23	Gem Premier 3000	Mindray BS120	Serum (coccygeal v.)	Hepar-WB (coccygeal v.)	5.70#	28.9/–17.4	NR	No error	< 40.0	NR	28.6	98.2	0.63
23	Edan i15Vet	Mindray BS120	Serum (coccygeal v.)	Hepar-WB (coccygeal v.)	–10.90#	12.4/–34.1	NR	No error	≥ 60	NR	31.3	94.7	0.63
23	Gem Premier 3000	Mindray BS120	Serum (coccygeal v.)	Hepar-WB (coccygeal v.)	5.70#	28.9/–17.4	NR	No error	≥ 60	NR	89.6	70.7	0.80
47	POC (I.B Co., Ltd., Tokyo)	Catachem Well-T	Hepar-plasma	Hepar-WB	–5.84	5.18*	4.45	NR	NR	NR	NR	NR	NR
56	Accu-Chek Aviva Plus	AU5800, Beckman Coulter	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	11.70	26.46/–3.24	2.20	No error	NR	NR	NR	NR	NR
56	Aga Matrix	AU5800, Beckman Coulter	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	5.22	32.76/–22.5	6.20	Proportional error	NR	NR	NR	NR	NR
56	Contour Next	AU5800, Beckman Coulter	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	–10.8	10.44/–23.58	4.00	No error	NR	NR	NR	NR	NR
56	FreeStyle Precision Neo	AU5800, Beckman Coulter	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	–4.50	11.7/–20.7	5.60	No error	NR	NR	NR	NR	NR
56	Nova Max Plus	AU5800, Beckman Coulter	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	9.36	29.7/–10.8	6.70	No error	NR	NR	NR	NR	NR
56	Precision Xtra	AU5800, Beckman Coulter	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	5.94	24.66 to –12.78	4.70	Const./ propor. errors	NR	NR	NR	NR	NR
98	Precision Xtra	Erba XL-200	Serum (coccygeal v.)	WB (coccygeal v.)	1.26	5.40/–8.10	3.10	No error	NR	NR	NR	NR	NR
60	FreeStyle Precision	Cobas 6000/c501	Plasma (NaF) (coccygeal v.)	Capillary WB (vulva skin)	–18.80	7.96*	5.20	Const./ propor. errors	≥ 60	43.0	76.0	80.0	87.4
60	GlucoMen LX Plus	Cobas 6000/c502	Plasma (NaF) (coccygeal v.)	Capillary WB (vulva skin)	20.80	15.41*	14.40	Const./ propor. errors	≥ 60	49.0	92.0	85.0	93.4
60	WellionVet GLUCO	Cobas 6000/c503	Plasma (NaF) (coccygeal v.)	Capillary WB (vulva skin)	–11.2	6.42*	15.70	Const./ propor. errors	≥ 60	95.0	39.0	92.0	70.4
60	FreeStyle Precision	Cobas 6000/c501	Plasma (NaF) (coccygeal v.)	WB with NaF (coccygeal v.)	–4.89	6.31*	5.20	Const./ propor. errors	≥ 60	59.0	75.3	91.4	91.7
60	GlucoMen LX Plus	Cobas 6000/c502	Plasma (NaF) (coccygeal v.)	WB with NaF (coccygeal v.)	24.64	15.19*	14.40	Proportional error	≥ 60	49.0	85.4	69.5	85.2
60	WellionVet GLUCO	Cobas 6000/c503	Plasma (NaF) (coccygeal v.)	WB with NaF (coccygeal v.)	–9.98	6.67*	15.70	Const./ propor. errors	≥ 60	94.0	55.1	84.8	70.0
65	Precision Xtra	AU680, Beckman Coulter	Hepar-plasma (coccygeal v.)	Hepar-WB (coccygeal v.)	–17.00	–22.00/–11.00	5.50	Const./ propor. errors	NR	NR	NR	NR	NR

Ref.	POCA	RM device or kit	Blood sample for RM	Blood sample for POCA	MB of RM-POCA (mg/dl)	LA or (±) SD* of MB (mg/dl)	IA-CVM of POCA (%)	Regression (PB/Deming)	BGC-TRH for RM (mg/dl)	O-TRH for POCA (mg/dl)	SE (%)	SP (%)	AUC
57	FreeStyle Precision Neo	Hitachi 911	Serum (coccygeal v.)	WB (coccygeal v.)	DNA	DP	1.7	NR	< 45	NR	91.0	46.0	NR
57	FreeStyle Precision Neo	Hitachi 911	Serum (coccygeal v.)	WB (coccygeal v.)	DNA	DP	1.7	NR	45-63	NR	41.0	79.0	NR
57	FreeStyle Precision Neo	Hitachi 911	Serum (coccygeal v.)	WB (coccygeal v.)	DNA	DP	1.7	NR	≥ 65	NR	16.0	100.0	NR
95**	Precision Xtra	Cobas c311	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	–0.54	19.98/–20.88	2.40	Const./ propor. errors	1.05***	NR	84.0	52.0	NR
95**	Precision Xtra	Cobas c312	Plasma (NaF/C2K2O4) (coccygeal v.)	WB (coccygeal v.)	–0.54	19.98/–20.89	2.40	Const./ propor. errors	1.15***	NR	69.0	85.0	NR

Explanations: Ref. – reference number; RM – reference method; IA-CVM – intra-assay coefficient of variation for medium concentration; PB/Deming – Passing-Bablok/Deming regression equation; BGC-TRH – blood glucose concentration threshold for RM; O-TRH – optimized threshold for POCA; MB – mean bias by Bland-Altman plots; LA – limits of agreement by Bland-Altman plots; SD* – standard deviation; SE – sensitivity; SP – specificity; AUC – area under the curve; Coccygeal v. – coccygeal vessels; WB – whole blood; Hepar-WB – heparinized whole blood; DP – data not presented; NR – not reported (not calculated or no statistics performed); Const./propor. – constant and proportional; * – standard deviation (LA was not reported); ** – study based on glucose tolerance test (0.25 g/kg dextrose infusion and analysis of glucose before and 80 min later. Insulin resistance: ratio ≥ 1.05 or 1.15), *** – cut-off point of glucose tolerance test (glucose ratio before and 80 min after infusion); # – MB and LA refer to = POCA – RM (positive MB refers to overestimation, negative MB refers to underestimation); Remark – glucose concentration units were converted from mmol/L to mg/dl by multiplication with a factor of 18.

2.55% for TAE. According to the Royal College of Pathologists of Australasia Analytical Quality, the TAE for iCa should not exceed ± 0.05 mmol/L, and for tCa, it should not exceed ± 0.10 mmol/L for values less than 2.5 mmol/L and ± 4% for tCa greater than 2.5 mmol/L (92). Additionally, Westgard (92) recommended a TAE of 2% for iCa.

According to the Canadian University of Physicians and Surgeons of Saskatchewan, TAE for BHB was defined as two times the standard deviation. The American Association for Clinical Chemistry specifies a TAE of less than 0.3 mmol/L for BHB concentrations below 1 mmol/L and a TAE of less than 30% for BHB concentrations of 1 mmol/L or higher in humans (63). A TAE of 18.7% was also reported as a desirable bias for BHB based on biological variation (49).

Methodology and statistical analysis

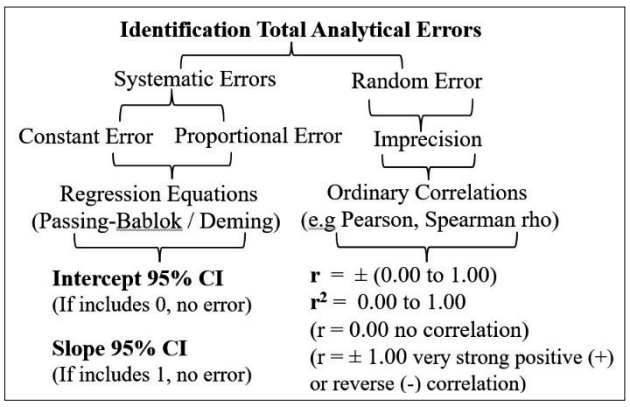
The number of samples. At least 40 samples with a wide range of concentrations should be analyzed for method comparison studies (7, 44). Method comparison studies require low, normal, and high concentrations of the parameter in question. A broad analytical range is very helpful in validation studies (65). Therefore, more than 40 samples may be needed to ensure sufficient coverage of all required concentrations at appropriate biological stages, which is an important part of the methodology. The transition period in dairy cows, where parameters fluctuate considerably, can be used to include sick and healthy animals. The calculation of sample size in prevalence studies has already been described in detail (13). The sample size can be calculated using the expected prevalence and a clinically acceptable width of a 95% CI to obtain realistic results. The results of studies with a small and unrepresentative number of samples may not be reliable, especially at a low prevalence. A total of 81 to 1300 samples were analyzed in the method comparison studies listed in Tables 1, 2, and 3. This is confusing in that, with a small sample size, it may not be possible to reliably identify sick and healthy animals. It is then possible that these will be random observations due to the overestimation or underestimation of POCA.

Statistical analysis for method comparison.

Agreement and interchangeability. A good agreement between two methods means that they can replace each other (interchangeability) when the same parameters are analyzed under the same conditions. The usual statistical tests for comparing means/standard deviations between two methods (paired or independent samples t-tests, Wilcoxon signed rank test) were not recommended for method comparison studies, because they do not analyze proportional differences (7, 44, 54). However, the mean/standard deviation, median, minimum, and maximum of the analytes measured by each method are always useful to know, as they show the numerical value and trend of each method. When reviewing the studies in Tables 1, 2, and 3, different statistical analyses were used to validate the methods. These were the Passing-Bablok and Deming regression equations for determining constant and proportional

errors, the Bland-Altman agreement plot for the determination of the mean bias and limit of agreement with a 95% CI, Lin's concordance correlation coefficient (CCC), Cohen's kappa coefficient (CKC) for subjective and nominal values, and the ordinary correlation coefficient (the ordinary CC) (Pearson and Spearman rank) for linear and nonlinear monotonic relationships. There are indeed differences in the interpretation and choice of statistical analysis for the validation studies reviewed in the present study.

Regression equations of Passing-Bablok and Deming. Both regressions determine systematic errors, such as constant and proportional errors, and observe the degree of agreement between two methods (44, 54). This means that they analyze the measurement error for both the independent (x) and the dependent (y) variables (7, 44, 54). The constant systematic error indicates that one method consistently yields higher or lower values than the other method. The proportional error indicates that differences between the two methods are proportional to the magnitude of the measurements (44). A perfect match means that the fitted regression hides the 45-degree line (y = x) (7, 44). The constant and proportional errors were specified with a 95% CI for the intercept and slope, respectively. In this case, perfect agreement between the two methods is indicated by an intercept of 0 and a slope of 1. The intercept and slope should not differ significantly from 0 and 1, respectively. If there are no constant and proportional errors, the two methods can be used interchangeably (7). If the 95% CI of the intercept includes 0, there is no significant constant bias. If it does not include 0, the two methods differ by at least one constant concentration of the analyzed parameter. For the slope, it was also required that there is no bias if 1 is included in the 95% CI. If 1 is not included, there is at least a proportional difference between the two methods. The regression equations of Passing-Bablok and Deming are reliable compared to the ordinary regressions in method comparisons, as both of them assume constant and proportional errors, rather than random errors (7, 44). Constant and proportional bias between two methods can be estimated and corrected (7). Both were often used in validation studies instead of the ordinary CC, but some studies did not use them (Tab. 1, 2, and 3).



The agreement plot of Bland-Altman. The Bland-Altman plot of agreement is a standard statistical analysis used in most studies for method comparisons (Tab. 1, 2, and 3). It was used to determine the negative or positive mean bias and limits of agreement with a 95% CI as the mean difference (± 1.96 standard deviation). The 95% limits of agreement represent the range of differences that contain 95% of future measurements. A good agreement can be supported by smaller limits of agreement with differences between two methods close to the mean difference, which should be very close to zero bias (9, 32) and should not differ significantly from the zero-mean difference. The Bland-Altman plot estimates an interval of agreement by considering the mean differences between two methods, and 95% of these differences should fall within the 95% confidence interval in Bland-Altman plots, indicating strong agreement (8, 32). The positive or negative mean bias, including the 95% limits of agreement, must be interpreted in terms of clinical relevance (32). A positive or negative mean bias of 0.05 mmol/L in the analysis of iCa, BHB, and glucose by a new test method compared to RM may be significant for iCa analysis, but not for BHB or glucose analysis. This mean bias may influence the interpretation of the diagnosis based on the threshold values presented in Tables 1, 2 and 3. Therefore, the device-specific positive or negative mean bias compared to RM must be taken into account. If the mean bias (mean bias = mean of RM – mean of POCA) of the new test method is negative, this indicates an overestimation; if it is positive, this indicates an underestimation by the POCA.

The concordance correlation coefficient (ρc, CCC). CCC has been used to assess agreement between observers or instruments for continuous variables (1, 16, 53, 88). CCC is frequently used in veterinary medicine to compare methods (47, 56, 57, 72, 75). CCC is a measure of how well bivariate pairs of observations agree compared to a gold standard, and it measures both precision and accuracy (1, 53, 57). CCC quantifies agreement between methods on a scale from –1 to +1 (–1: perfect disagreement, 0: independent and +1: perfect agreement) (16, 20). This was suggested when the data were not predefined for total errors (42). For interpretation of agreement, CCC was categorized as perfect (> 0.99), poor (< 0.90), moderate (from 0.90 to 0.95), and substantial (from 0.95 to 0.99) (1). However, a CCC of 0.81 was considered as almost perfect performance (44), 0.92 was interpreted as a very high degree of agreement and 0.56 as a moderate level of agreement (16). The limitations of CCC in evaluating model accuracy have also been reported (90). The categorization of CCC for interpretations has been done differently in different studies and by different researchers. There may still be need to categorize CCC for interpretation in different sciences, models, or data sets.

Cohen's kappa coefficient (K, CKC). It is a statistical method used to measure inter-rater reliability.

However, only some studies (48, 57, 95) used CKC to compare methods in veterinary medicine. CKC measures a range from –1 to +1, where 0.0 represents the degree of agreement to be expected by chance and 1.0 represents perfect agreement between the raters (62). CKC is a measure of agreement between two dependent categorical samples (judgments or observations). It considers the possibility of random agreement between the raters. However, CKC cannot be estimated when subjects' responses are not distributed across all valid response categories, and it was used when judgments are subjective, and categories are nominal. The strength of agreement according to CKC was originally interpreted by the inventor, but has been updated to recommend a different categorization, so CKC should be interpreted with caution in medicine, according to McHugh (62).

Cohen's Kappa Coefficient (CKC)

CKC	Agreement Level	Reliability of Data (%)
0.00 - 0.20	None	0 - 4
0.21 - 0.39	Minimal	4 - 15
0.40 - 0.59	Weak	15 - 35
0.60 - 0.79	Moderate	35 - 63
0.80 - 0.90	Strong	63 - 81
>0.90	Almost perfect	82 - 100

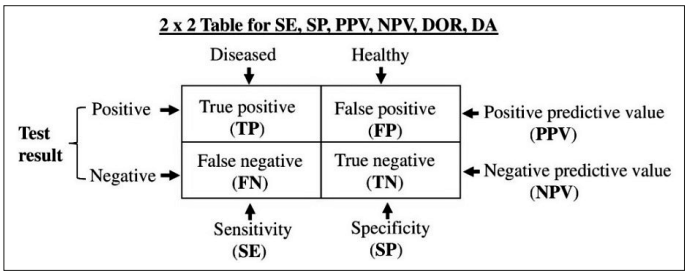
The ordinary correlation coefficient (CC). The ordinary CC (Pearson and Spearman rho) was frequently used in validation studies of POCAs (40, 48, 51, 76). The Pearson CC (parametric data distribution, linear relationship) and the Spearman CC (non-parametric data distribution, non-linear monotonic relationship) are measures of a relationship between two variables (1, 77). Both are used to determine CC (r), which is scaled from –1.0 to +1.0, where 0 indicates that there is no linear or monotonic relationship (77). The ordinary CC indicates a positive relationship when both variables are increasing in the same direction. If one variable is increasing, while the other is decreasing, it is a negative or inverse correlation (93). Since linear correlation is based on random measurement errors (imprecision) of the dependent variable, it is assumed that only Y measurements are associated with random measurement errors. The imprecision depends only on the distribution of random errors under given conditions (observed by the same method on similar test material under the same conditions) and does not refer to the true value (44). Even a small CC (e.g., r = 0.25) between two variables can be statistically significant ($P < 0.05$). In a large data set, a small but significant CC should not be mistaken for a clinically relevant correlation (77). It should be noted that even a high, significant, and positive CC does not indicate good agreement between two different methods, but only a relationship (32, 44). The ordinary CC between two methods can always mislead and is therefore not to be favored when comparing methods for a decision; it is not a measure of acceptance (32, 44, 57). However, if r is > 0.975 or > 0.99, the ordinary CC may provide useful information about errors. A constant error is present if the intercept differs significantly from 0, while a proportional error exists if the slope differs from 1. If r < 0.975 or 0.99, the data must be analyzed differently, or an alternative regression analysis, such as the Passing-Bablok or Deming regression equations, may be required (44), as these consider the accuracy and precision of a measurement for continuous variables (7, 44). Moreover, the interpretation of the ordinary CC may vary between different sciences (politics, medicine, and psychology) (1) and different studies (77). Westgard (93) and Schober et al. (77) categorized the ordinary CC according to how strongly two variables are related: the correlation strength of r = 0.00 to ± 1.00.

Strength of Correlation Coefficient (r)

Westgard (93)		Schober et al. (77)	
r	Interpretation	r	Interpretation
0.90 to 1.00	Very high	0.90 to 1.00	Very strong
0.70 to 0.89	High	0.70 to 0.89	Strong
0.50 to 0.69	Moderate	0.40 to 0.69	Moderate
0.30 to 0.49	Low	0.10 to 0.39	Weak
0.00 to 0.29	Little	0.00 to 0.10	Negligible

The paradox between agreement and diagnostic accuracy. A POCA may have acceptable diagnostic accuracy for a disease based on a predetermined threshold of the parameter set for RM, even if the agreement and interchangeability between RM and the POCA are poor due to a positive or negative bias and constant or proportional errors. It is then substantially better when an optimized threshold for the POCA is used (5, 22, 83). The agreement or interchangeability of a POCA with RM and the diagnostic accuracy results of the POCA should be interpreted separately because of the impact of disease prevalence and the threshold of parameters (e.g., BHB, iCa, and tCa) on diagnostic accuracy. The 95% CI of the mean bias needs to be considered and clinically interpreted.

The assessment of diagnostic accuracy. The identification of genuinely sick and healthy individuals according to RM in a population can be carried out using a 2 × 2 contingency table (33, 87). For this purpose, the identification of true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) results of the test are required. The 2 × 2 contingency table can be used to calculate the SE, SP, PPV, NPV, diagnostic odds ratio (DOR), and diagnostic accuracy (DA) of POCAs.



The evaluation of the diagnostic performance of a POCA compared to RM requires a predetermined threshold (cut-off point, disease classifier) for biochemical parameters for a given disease, such as BHB ≥ 1.20 mmol/L for SCK, iCa < 1.00 mmol/L and tCa < 2.15 mmol/L for SCH or BGC < 40 mg/dl for hypoglycemia. The receiver operating characteristics (ROC) analysis is a technique for visualizing, organizing, and selecting an optimized threshold based on their performance (27). The ROC analysis has been used to determine SE, SP, AUC, PPV, NPV, Youden index (*J*), and an optimized cut-off point of POCAs (22, 27, 42, 60, 85). Optimized cut-off points for a POCA determined by the ROC analysis corresponds to the highest SE and SP of the threshold value of RM, i.e. the threshold value with the highest *J*. *J* is calculated as *J* = SE (%) + SP (%) – 100 (36, 42, 85). However, in Tables 1, 2, and 3, SE and SP are preferred for evaluating the diagnostic performance of POCAs, while the optimized threshold and AUC are often omitted.

The true positive rate (sensitivity, SE) and the true negative rate (specificity, SP). The SE and SP of a POCA indicate the proportion of diseased and healthy individuals in a population, respectively (33, 87). It is assumed that SE and SP are independent of the prevalence of the disease, but depend on the spectrum of the disease (85). Others, however, (50, 67) reported that SE and SP may be influenced by the prevalence of the disease. Higher prevalence was associated with a higher estimated SE and a lower estimated SP (67). The use of SE and SP as parameters for evaluating diagnostic accuracy can lead to problems, as these depend on a diagnostic criterion (cut-off points) (37). SE and SP should always be interpreted together with thresholds established for the classification of the disease. Dairy cows exhibit fluctuating concentrations of BHB, tCa, iCa, and BGC during the transition period (18, 23, 51, 57, 70), which affect the prevalence of the associated disease, SE and SP depending on the time of blood sampling and the cut-off points of the parameters. Diurnal variations should also be considered (75). The SE and SP of POCAs can be calculated using TP, TN, FP, and FN (87). A combined SE and SP of at least 180% with an AUC of > 0.80 would be desirable.

Calculation of Sensitivity (SE) and Specificity (SP)

$$SE\ (\%) = \frac{\text{True positives}}{\text{True positives + False negatives}} \times 100$$

$$SP\ (\%) = \frac{\text{True negatives}}{\text{True negatives + False positives}} \times 100$$

The positive predictive value (PPV) and the negative predictive value (NPV). These are more important than SE and SP when screening a population for disease. Studies need to provide insights into SE, SP, PPV, and NPV when interpreting screening test results (87). PPV is the probability of being ill if the test is positive, and NPV is the probability of being healthy if the test is negative. PPV and NPV are influenced by the prevalence of the disease. PPV increases with a higher prevalence of the disease, while NPV decreases with a higher prevalence (37). PPV and NPV can be calculated using TP, TN, FP, and FN (27, 87).

Calculation of Positive and Negative Predictive Values (PPV, NPV)

$$PPV\ (\%) = \frac{\text{True positives}}{\text{True positives + False positives}} \times 100$$

$$NPV\ (\%) = \frac{\text{True negative}}{\text{True negatives + False negatives}} \times 100$$

The area under the curve (AUC). The AUC of the ROC analysis indicates the overall diagnostic accuracy of the method. Perfect accuracy, with an AUC of 1.00 (100%), means that the test is perfect in distinguishing between diseased and non-diseased individuals, whereas an AUC of 0.50 means that it is worthless (37, 96). AUC indicates how high the discriminatory power of a test method is (85). An identical AUC can lead to different SE and SP values because of different predetermined thresholds for RM (22, 85). Since SE, SP, PPV, and NPV depend on the prevalence of the disease, AUC indicates the overall DA of a POCA, as it considers both SE and SP. The overall DA of a test was categorized based on the AUC rate. The interpretation of AUC varies slightly in the different reports depending on categories (31, 85, 96). AUC was often neglected in reporting ROC analyses (Tab. 1, 2, and 3).

Interpretation of Area Under the Curve (AUC)

AUC	Diagnostic Accuracy
> 0.90 ≤ 1.00	Excellent (91 – 100 %)
> 0.80 ≤ 0.90	Very good (81 – 90 %)
> 0.70 ≤ 0.80	Good (71 – 80 %)
> 0.60 ≤ 0.70	Acceptable (61 – 70 %)
> 0.50 ≤ 0.60	Poor (51 – 60 %)
= 0.50	Worthless

Diagnostic accuracy (DA). DA is a measure of the effectiveness of the test. The accuracy of a test is its ability to correctly distinguish patients from healthy individuals (85). DA depends on the prevalence of the disease (85). To determine the accuracy of a new test, the proportions of TP, TN, FP and FN must be calculated mathematically.

Calculation of Diagnostic Accuracy (DA)

$$DA\ (\%) = \frac{TP + TN}{TP + TN + FP + FN} \times 100$$

The diagnostic odds ratio (DOR). DOR is a global measure of test performance. It combines device performance (SE + SP) as a prevalence-independent indicator with the advantage of accuracy as a single indicator (33, 85). DOR ranges from 0 to infinity, with a higher DOR indicating better discriminatory test performance. DOR increases steeply when SE or SP becomes nearly perfect (33). The DOR of a test

method is the ratio of the odds of positivity in subjects with disease relative to the odds in subjects without disease. DOR can be calculated using SE and SP or TP, TN, FP, and FN (33, 85).

Calculation of Diagnostic Odds Ratio (DOR)

$$DOR = \frac{\text{Sensitivity}}{\text{1- Sensitivity}} \Big/ \frac{\text{1- Specificity}}{\text{Specificity}}$$

$$DOR = \frac{\text{True positives}}{\text{False negatives}} \Big/ \frac{\text{False positives}}{\text{True negatives}}$$

The odds, odds ratio, and relative risk. The odds can be calculated by dividing TP (diseased) by TN (not diseased): odds = TP (diseased)/TN (healthy). The odds can be between 0.0 and infinity depending on sample size (10, 35, 69). The odds can increase exponentially from a prevalence of 90%, depending on sample size. Exactly the same prevalence rate always leads to exactly the same odds. The odds ratio (OR) is a ratio of the odds of one group to the odds of another group. OR indicates the probability of a disease or event in a group exposed to the disease compared to another group (10, 84). OR ranges from zero to infinity (35). If OR is 1.00, exposure has no effect on the probability of the outcome between groups, but exposure is associated with a higher probability of the outcome if OR > 1.0 or a lower probability of the outcome if OR < 1.0 (84). OR between two groups is significant if a 95% CI does not include 1.0 (69). Bland and Altman (10) recommend chi-square tests (X^2) using a 2 × 2 contingency table or discussion using multivariable logistic regression. The interpretation of OR and relative risk should not be mixed as they are calculated differently. The relative risk is the ratio of prevalence rates between two groups exposed to a disease or event (10, 69). OR exaggerates the effect of exposure compared to the relative risk (35). OR between two groups may reverse direction as the prevalence of the disease increases in the whole population.

Calculation of Odds Ratio (OR)

$$OR = \frac{\text{Odds of Group A}\ [\text{Number of TP (diseased) / Number of TN (healthy)}]}{\text{Odds of Group B}\ [\text{Number of TP (diseased) / Number of TN (healthy)}]}$$

The reverse multiple cut-off points (RMC) analysis. This hypothesis evaluates the diagnostic performance and accuracy of POCAs based on absolute positive prevalence (APP), true positive prevalence (TPP), and BGCs at decreasing (BGC < 40 to < 60 mg/dl) and increasing (BGC ≥ 40 to ≥ 60 mg/dl) cut-off points (DCP and ICP, respectively), including at different stages of lactation (23). RMC analyzes differences in APP, TPP, BGC, and AUC between the POCA and RM using acceptance limits of ± 1.0% and 0.0% (excellent), ± 2.5% and ± 1.0% (fairly good), ± 2.5% and ± 5% (good), ± 5% and ± 10% (moderate), ± 10% and ± 15% (low), and > 15% (unacceptable). RMC can be used to determine how POCAs respond at low and high BGCs to diagnose hypoglycemia and hyperglycemia during the transition period, as it has been speculated that the Bland-Altman plots of agreement and the Passing-Bablok regression equation respond differently at low and high BGCs during the transition period. Although the RMC method is based on the categorization of agreement and diagnostic accuracy, it provides differences for BGC, AUC, APP, and TPP at DCP and ICP and shows overall diagnostic agreement with RM (23).

The analysis of BHB, tCa, iCa, and BGC by POCAs

The analysis of BHB

An elevated blood BHB level is a marker for the diagnosis of hyperketonemia, such as ketosis and SCK (2, 4, 22, 78, 89). Ketosis and SCK are often associated with a decreased BGC (17, 89). POCAs are commonly used to analyze BHB in whole blood with and without anticoagulants or in serum and plasma (Tab. 1). A cut-off value of BHB ≥ 1.20 mmol/L in blood has become widely accepted, mainly because it has been associated with postpartum SCK-related disorders and decreased milk production (2, 22, 57, 81). Other thresholds have also been reported, as shown in Table 1. Some studies did not assess the accuracy of POCAs in diagnosing SCK through BHB analysis (49) or did not report regression equation results (5, 41, 48, 57, 59, 72, 76, 83), so the interchangeability with RM, as well as SE, SP, and AUC, remained unknown. Most POCAs were sufficiently and accurately able to analyze BHB with high SE, SP, and AUC in coccygeal whole blood and capillary veins, but the SP and AUC of POCAs were much lower in serum and EDTA plasma. As incidence and prevalence can vary by farm, region, season, dairy breed, parity, and country (2, 4, 22, 36, 84), it is advisable to test at-risk cows or routinely test all cows with an accurate POCA between days 5 and 10 post-calving and then again on day 14-20 post-calving.

The analysis of iCa and tCa

Both tCa and iCa are markers for monitoring hypocalcemia in dairy cows (51, 61). The tCa value is often determined by wet or dry chemical analysis in serum or plasma, which can be time consuming, but is recognized as RM (Tab. 2). The unit of tCa or iCa can be converted from mg/dL to mmol/L (mM) with a factor of 0.2495 (1 mg/dL tCa or iCa = 0.2495 mmol/L tCa or iCa). According to some authors, POCAs showed adequate SE, SP, and AUC in the analysis of tCa in serum (51), but others did not report diagnostic accuracy and regression equation results when using heparinized whole blood (47). The use of a tCa threshold for SCH diagnosis as RM for determining a corresponding iCa cut-off point has not been recommended in the ROC analysis due to low diagnostic accuracy (22), as the ratio between tCa and iCa fluctuates around calving and is unstable (18, 51). Different thresholds for tCa have

been used to define SCH: from tCa < 2.00 mmol/L to 2.20 mmol/L depending on days in milk (51, 61, 22, 68). A threshold of iCa < 1.00 mmol/L in whole blood has often been used to define SCH (22, 97). The diagnostic accuracy and validation of POCAs with blood gas analyzers have shown that SE, SP and AUC are sufficiently high (Tab. 2). However, in some studies, the diagnostic accuracy of POCAs was not investigated or reported (68, 82) – the device was only validated with RM, leaving SE, SP, AUC, PPV, and NPV unknown. The use of a blood gas analyzer as a POCA for the analysis of iCa is very common (Tab. 2). The measured iCa value is automatically corrected to a blood pH of 7.40 and displayed, as there is a significant negative correlation between blood iCa and pH. Blood gas analyzers measure iCa values in heparinized whole blood by the potentiometric method with an ion-selective electrode (19, 22). iCa is a physiologically active and diffusible form of tCa (18, 19, 22, 82), which means that a correct analysis of iCa makes it possible to correctly diagnose SCH for treatment and prevention.

The analysis of BGC

An elevated BHB level due to SCK has been associated with a reduced BGC (17, 89) and an increased concentration of non-esterified fatty acids (70, 78). Wet-chemistry analyzers measure BGC by the hexokinase or glucose oxidase method as RM and they are used in studies on the validation and diagnostic accuracy of POCAs (Tab. 3). BGC can be expressed in mg/dL or mmol/L. BGC (mg/dl) = 18 × BGC (mmol/L). BGC < 40 or < 45 mg/dL for hypoglycemia and ≥ 60 or ≥ 65 mg/dL for hyperglycemia (23, 57, 60) have been used in diagnostic accuracy studies of POCAs in dairy cows. BGC varies significantly between prepartum, calving, and early lactation (23, 70). Hyperglycemia (BGC ≥ 65 mg/dL) due to reduced insulin sensitivity is very common before calving (70) and at calving with a rate of 41.4% (23). Hypoglycemia (BGC < 45 mg/dL), on the other hand, is commonly observed within 4-9 days after calving at a rate of 45% (23) or at a rate of 32% between days 2 and 14 after calving (57) due to the onset of lactation. The effect of diurnal variation on BGC plays an important role in the analysis of evenly distributed samples with high, low or normal BGC in the study population. Some studies (Tab. 3) reported only the ordinary CC or CCC of POCAs with RM in cows, but not the diagnostic accuracy for hypoglycemia or hyperglycemia (47, 56, 98). The SE, SP, and AUC values of POCAs were not sufficiently high (Tab. 3) for detecting hypoglycemia or hyperglycemia in dairy cows, which could have been due to the effects of hematocrit, total protein, and anticoagulants on BGC, as these were not considered. Voyvoda and Erdogan (89) reported an agreement between Optium Xceed and RM in dairy cows with an overestimation of 4.86 mg/dL (converted from mM) by Optium Xceed, but the diagnostic performance was not reported. Karapınar et al (46) found a mean underestimation of –0.60 mg/dL, systematic and constant errors in the regression equation for Freestyle Optimum Neo in jugular whole blood compared to RM in heparinized plasma in sick calves, and high SE, SP, and AUC were observed after correction of BGC for hematocrit, total protein, and age of calves.

Critical analysis of the studies on POCAs

The concentrations of BHB, tCa, iCa, and BGC were frequently analyzed with different POCAs in whole blood, serum, and plasma from the coccygeal/jugular vessels and capillaries for validation and diagnostic accuracy studies. The results for agreement with RM and intra-assay CV varied probably due to biological and external factors and the number of samples, although the same POCAs were used in some of the studies. The use of ordinary CC in some validation studies does not show constant and proportional systematic errors with a 95% CI, but only random errors, and the interpretation of ordinary CC varies between different sciences and researchers. Moreover, many studies performed CCC, which quantifies agreement and provides *pc*, but the interpretation of *pc* varied between studies, and limitations were also mentioned. In addition, CKC was also performed for agreement, but it quantifies the degree of agreement between two nominal variables and categorizes them. It could be applicable to subjective decisions/observations and categories that are nominal. In some validation studies, there was no information on the assessment of the diagnostic performance and precision of POCAs, although POCAs may have sufficient diagnostic accuracy despite disagreement with RM. The SE and SP of POCAs were always reported in the studies. However, AUC values were not preferentially reported in some of these studies, although AUC is a global diagnostic measure that includes both SE and SP in the calculation. On the other hand, diagnostic performance may vary depending on the site of sample collection, the type of samples, and the cut-off points of parameters affecting prevalence. It is advisable that validation studies of POCAs be accompanied by a verification of their diagnostic accuracy, including SE, SP, optimized cut-off points, AUC, PPV, and NPV, compared to RM at a specific anatomical site.

Overall conclusion

Accurate diagnosis with POCAs is essential for rapid prevention and treatment of SCK and SCH. The studies showed that the diagnostic performance of POCAs was sufficiently good to diagnose SCK and SCH by analyzing BHB, tCa, and iCa, although inconsistencies concerning the high mean bias with regard to RM were observed, and no results for systematic errors, CV, and TOE were reported. On the other hand, the analysis of BGC by POCAs yielded inadequate results, probably due to the influence of hematocrit, total protein in whole blood, and anticoagulants in plasma or the use

of serum analysis as RM. The BGC analysis by POCAs remains a point of discussion in humans and animals because of differences in glucose dissolution between erythrocytes and plasma, as well as the influence of hematocrit and anticoagulants. Studies using the same POCAs may yield different results because the effects of external, biological, and methodological factors are often overlooked. The use of validated POCAs with adequate diagnostic performance (AUC > 0.80, SE > 90%, SP > 90% to avoid unwanted false positive and false negative results) can, under optimal conditions, replace laboratory methods for analyzing BHB, tCa, and iCa in dairy cows. However, it is useful to know the mean bias with regard to RM, as the probability of detecting hyper-concentration or hypo-concentration associated with the threshold may increase due to a positive mean bias or a negative mean bias, respectively. Finally, the standardization of data reporting and methodology in the validation and diagnostic accuracy studies of POCAs is recommended, considering external, methodological, and biological factors.

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