Method comparison and validation of a prototype device for measurement of ionized calcium concentrations cow-side against a point-of-care instrument and a benchtop blood-gas analyzer reference method

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ABSTRACT

The objective of this study was to assess an optimized ion-selective electrode Ca-module prototype as a potential cow-side device for ionized Ca (iCa) measurements in bovine blood. A linearity experiment showed no deviation from linearity over a range of iCa concentrations compared with a commercial point-of-care (POC) device commonly used in the field (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA) and a laboratory gold standard benchtop blood-gas analyzer [reference analyzer (RA); ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark]. Coefficient of variation on 3 samples with high, within-range, and low iCa concentrations ranged from 1.0 to 3.9% for the prototype. A follow-up validation experiment was performed, in which our objectives were to (1) assess the performance of the prototype cow-side against the POC_{VS} (farm gold-standard) using fresh non-anticoagulated whole-blood samples; (2) assess the performance of the prototype and the POC_{VS} against the RA in a diagnostic laboratory using blood collected in a heparin-balanced syringe; and (3) assess the agreement of the prototype and POC_{VS} on-farm (fresh non-anticoagulated whole blood) against the RA on heparin-balanced blood. Finally, sensitivity and specificity of the results obtained by the prototype and the POC_{VS} cow-side compared with the results obtained by the laboratory RA using 3 different iCa cut points for classification of subclinical hypocalcemia were calculated. A total of 101 periparturient Holstein cows from 3 dairy farms in New York State were used for the second experiment. Ionized Ca results from the prototype cow-side were, on average, 0.06 mmol/L higher than the POC_{VS}. With heparin-balanced samples under laboratory conditions, the prototype and POC_{VS} measured an average 0.04 mmol/L higher and lower, respectively, compared with the RA. Results from the prototype and POC_{VS} cow-

side were 0.01 mmol/L higher and 0.05 mmol/L lower, respectively, compared with results from the laboratory RA on heparinized blood. Sensitivity and specificity for the prototype and the POC_{VS} under farm conditions at 3 potential subclinical hypocalcemia cut points were 100 and $\geq 93.5\%$, respectively. This novel ion-selective electrode Ca-module could become a rapid low-cost tool for assessing iCa cow-side, while qualitatively allowing classification of subclinical hypocalcemia on-farm.

Key words: ionized calcium, subclinical hypocalcemia, point-of-care, cow-side

INTRODUCTION

One of the challenges for the periparturient dairy cow is the maintenance of ideal blood Ca concentrations to support milk production and immune function. Subclinical hypocalcemia (**SCH**) is a prevalent condition afflicting approximately 50% of multiparous dairy cows in the early postpartum period (Reinhardt et al., 2011), and is a disorder being characterized by various research groups. Therefore, measurement of Ca to assess individual animal calcemic status and optimize preventative strategies for SCH is important.

Calcium is found in 3 forms in the blood: (1) protein-bound, (2) complexed to proteins and anions, and (3) ionized (recognized as the biologically active form). Both total Ca (**tCa**; overall measurement of the 3 fractions) and ionized Ca (**iCa**) can be measured in the laboratory, albeit using different techniques (i.e., dye-binding methods for tCa and direct potentiometry for iCa). Total Ca measurement is easy to perform, readily available in most laboratories, cheaper, and considered more stable with storage than iCa (Forman and Lorenzo, 1991). In contrast, iCa measurements are more expensive and affected by changes in pH, and, consequently, unstable with storage (Burrit, 1993). Although approximately 50% of tCa is thought to be in the ionized form, tCa cannot always reliably predict iCa concentrations, particularly with changes in pH (Wang et al., 2002; Lam et al., 2013). In addition, tCa, but not iCa, is affected by changes in albumin concentration,

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such as that due to hydration status. As the dairy cow approaches parturition and begins lactation, normal physiological changes occur in albumin concentrations (Piccione et al., 2011), which can affect tCa measurements but not iCa. In addition, the transition to lactation is a high-risk period for an altered hydration status as well as electrolyte imbalances. This means that tCa may not be the ideal test for monitoring herds for SCH and iCa may be of higher diagnostic value.

The introduction of ion-selective electrode (ISE) technology in clinical medicine has allowed for the direct measurements of iCa in blood, serum, and plasma. Testing is usually done in clinical pathology laboratories using dedicated blood-gas instrumentation equipped with a Ca-specific ISE; however, the logistical difficulty of getting field samples to a laboratory decreases the availability of routine iCa testing. Currently, 1 point-of-care (POC) device that employs direct potentiometry (POC_{VS}; VetScan i-STAT; Abaxis North America, Union City, CA) is commonly used by in-hospital dairy clinicians, consultants, and researchers for assessing individual animal iCa status. The average cost of the simplest POC_{VS} cartridge that offers iCa measurement (CG8⁺) is approximately \$17.50, which substantially limits common use of this device in the field. Moreover, results for a single test-cartridge take 1.5 min to output, adding to the time and cost of analysis. To the best of our knowledge, only 1 study has evaluated the performance of the POC_{VS} in bovine blood (Peiró et al., 2010); those authors only tested 24 blood samples from 24 clinically healthy individuals and found that the limits of agreement as compared with a benchtop blood-gas analyzer was nearly ± 0.1 mmol/L.

Due to the aforementioned limitations of iCa testing for SCH mitigation strategies at the individual cow level, the monitoring of herd-level SCH and implementation of preventative strategies would be more successful if low-cost iCa testing could be done on the farm using a portable device. Our group has been working on the optimization of a low-cost, rapid test ISE Ca-module prototype in conjunction with engineers of HORIBA Advanced Techno (Kyoto, Japan), to deliver an instrument that enables measurement of iCa cow-side in a rapid fashion.

Our study had several objectives over 2 experiments. For experiment 1, our objectives were to (1) assess the linearity of the optimized prototype using a range of iCa concentrations prepared from varying dilutions of a high- and low-Ca heparinized blood samples against the commercial POC_{VS} device (farm fold standard) and a reference analyzer (RA; laboratory gold-standard; ABL-800 FLEX; Radiometer Medical, Copenhagen, Denmark) at the New York State Animal Health Diagnostic Center (AHDC; Ithaca, NY); and (2) determine

the within-run imprecision for the optimized prototype. Experiment 2 was designed to test the prototype under field and laboratory conditions and our objectives were to (1) assess method agreement of the optimized prototype as a cow-side test against the POC_{VS} in bovine fresh whole blood (i.e., intended use of both instruments); (2) assess method agreement between the prototype and the POC_{VS} under laboratory conditions against the RA using heparinized whole blood collected in heparin-balanced syringes; and (3) assess method agreement and the sensitivity (Sn) and specificity (Sp) between the results of the prototype and the POC_{VS} obtained cow-side with fresh whole blood against the RA based on heparinized whole blood using 3 potential iCa cut points for SCH classification (≤ 0.95 , 1.00, and 1.05 mmol/L).

MATERIALS AND METHODS

All procedures used for blood collection and animal handling were reviewed and approved by Cornell University's Institutional Animal Care and Use Committee (protocol 2014-0105).

Instrument Optimization

A series of laboratory tests were performed from 2014 to 2017, in conjunction with company engineers (HORIBA Advanced Techno), to optimize the accuracy and reproducibility of an ISE Ca-module prototype for potential use as a cow-side test. An optimized prototype was developed after testing and manipulation of a series of trial prototypes that underwent significant modifications. Briefly, software changes to alter measurement units and resolution were performed on a commercially available ISE Ca-module, which is marketed for food, soil, and water sample use (B-751 LAQUAtwin; HORIBA Advanced Techno). Next, calibration set points were modified across prototype generations. Between-instrument imprecision in the second and third prototype generations were influenced by the inaccuracy of the temperature sensor. After ablation of the temperature-correction function employed in the differential potential algorithm of the instrument software, combined with improved results of blood measurement simulations having the electric potential corrected to 37°C, follow-up work on alterations of the device to minimize sample temperature effects took place. The most current and final prototype employs an optimized electrode for use in blood, and it was designed to allow for rapid iCa measurement on-farm (i.e., within 15 s of sample contact time with the sensor) by employing a proprietary differential potential cut point. The evaluation of the prototype under labo-

ratory conditions standardized the measurement time to 60 s, as it allowed for a better stabilization of the electric potential in heparinized samples based in work we established before initiation of this study (data not shown).

Ultimately, a series of tests were undertaken to find candidate calibration standards that would minimize the bias in measurements according to the most prevalent interfering cations found in blood (e.g., Na^+ and K^+), while including or not including adjustments for calibrator conductivities. A within-normal range (1.25 mmol/L; Radiometer Medical) and a high-iCa standard solution (5.0 mmol/L; Radiometer Medical) were used to perform a 2-point calibration before every measurement. In dairy cows, iCa has been shown to vary between 0.95 to 1.31 mmol/L (Lincoln and Lane, 1990).

Calibration Guidelines

Calibration protocols for the prototype were instituted based on prior knowledge of the first author. The methodology employed attempted to maintain ease of use in preparation for commercialization of the prototype and were (1) dispensing 0.5 to 0.7 mL of the within-normal range iCa standard solution (1.25 mmol/L) to the sensor while allowing 15 s of contact time before the calibration for a zero potential; (2) rinsing the electrode region with deionized water followed by gentle removal of residual water with delicate task wipes; (3) application of 0.5 to 0.7 mL of the high-iCa standard solution (5.0 mmol/L) followed by immediate calibration for the slope; (4) rinsing and moisture removal as per step 2; and (5) application of approximately 0.7 mL of blood for measurement. After blood measurement, the sensor was cleaned by rinsing the electrode area with deionized water, followed by 10 s of contact time with 0.5 to 0.7 mL of a 4% sodium hypochlorite solution on the sensor, then rinsing again with deionized water. Sodium hypochlorite solution was used to avoid potential protein coating of the ISE response membrane that could interfere with calibration and measurement performance.

Experiment 1

To prepare blood samples with varying iCa concentrations, a total of 200 mL of blood was collected at the Cornell University Teaching Dairy Barn (College of Veterinary Medicine, Ithaca, NY) using a 20-gauge $\times$ 3.8-cm Vacutainer needle from the right jugular vein of a recently fresh multiparous Holstein dairy cow into 10-mL lithium heparin Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) immediately before and 5 min after an intravenous 500-mL infusion of a 23% Ca glu-

conate solution (Agri Laboratories Ltd., Saint Joseph, MO). Blood samples were transported immediately to the AHDC laboratory for preparation of dilutions. The sample collected before 23% Ca gluconate infusion was diluted by a maximum of 10% using 0.9% sterile saline solution to create a low-iCa pool. This low-iCa pool sample was mixed at different ratios with the sample collected after the Ca infusion (high-iCa pool) to provide intermediate iCa concentrations. This method follows guidelines of linearity assessment provided by the Clinical and Laboratory Standards Institute (CLSI) in clinical blood chemistry studies (CLSI, 2013). As recommended by CLSI, triplicate measurements of the blood mixtures were performed side-by-side on the method under evaluation (i.e., the prototype) and once for the POC_{VS} and the RA in a randomized fashion. As the 2 latter methods standardize the temperature of the samples to 37°C with an internal heating block system before analysis, the samples were analyzed at 37°C in the prototype to allow for more objective comparison between methods. Blood samples were warmed to 37°C by placing it in a dry heat incubator for 20 to 30 min before analysis, whereas the standards used to calibrate the prototype were kept at room temperature (approximately 22°C). Short-term imprecision of the prototype was measured by performing 10 consecutive measurements in a sample with a low, within-normal range, and high iCa concentration, which were selected from the linearity experiment (Westgard, 2008).

Experiment 2

A total of 101 cows were enrolled in experiment 2 from 3 dairy farms in New York State over a period of 4 wk between May and June 2017. Samples were from cows between DIM 0 and 4 ($n = 76$), between DIM 5 and 12 ($n = 15$), and dry multiparous pregnant cows ($n = 10$). A total of 22% of the samples were from primiparous cows, with 35, 22, and 21% represented by parity 2, 3, and ≥ 4 , respectively. Blood samples were collected via coccygeal venipuncture using a 10-mL Vacutainer tube without additives (Becton Dickinson). Immediately after blood collection, approximately 2 mL of the sample was aspirated into a 6-mL polypropylene syringe and tested with the prototype and POC_{VS} cow-side as for objective 1. Then, 2 mL was aspirated into a 2-mL heparin-balanced syringe (PICO50; Radiometer Medical). The heparin-balanced syringes were then transported from the farm to the laboratory chilled inside a cooler with ice packs (approximately 4°C) and allowed to warm to room temperature (approximately 22°C) for 20 min before analysis on the prototype, POC_{VS} , and RA for objectives 2 and 3. Notably, these objectives were performed on heparinized blood samples, as

Figure 1. Flowchart outlining the logistics of blood sampling procedures and comparative analyses performed between ionized Ca measuring devices for experiment 2. Methods used were an ion-selective electrode (ISE) Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan), a commercial point-of-care device (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA), and a laboratory reference analyzer (RA; ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark).

non-anticoagulated whole blood could not be analyzed on the RA. The interval from blood collection at the farm until analyses in the laboratory was between 2:30 to 3:50 h for all samples. Figure 1 is an outline of the logistics of blood sampling procedures and comparative analyses performed. For the RA, quality control (QC) checks were performed daily to ensure measurement correctness.

Statistical Procedures

Descriptive statistics were performed using the FREQ, MEANS, and UNIVARIATE procedures of SAS version 9.4 (SAS Institute Inc., Cary, NC). Cumulative sum tests of linearity (from Passing and Bablok regressions, experiment 1), Bland-Altman plots, and Deming regressions (experiment 2) were performed in MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium). Evaluation of the within-run imprecision (CV) for the POC_{VS} and the RA were <1.0%. Sensitivity and Sp of the prototype to classify SCH at iCa concentrations ≤ 0.95 , 1.00, and 1.05 mmol/L were calculated using MedCalc Statistical Software. Data were graphed using GraphPad Prism version 7.03 (GraphPad Software, La Jolla, CA).

RESULTS

In experiment 1, cumulative sum tests of linearity from Passing and Bablok regressions showed that the prototype did not deviate from linearity against the POC_{VS} or the RA ($P = 0.63$ in both cases). Table 1 presents a summary of the linearity experiment. Coefficients of variation for the prototype obtained in a blood sample with a low (0.72 mmol/L), within-normal range (1.29 mmol/L), and high (2.0 mmol/L) iCa concentration were 3.9, 2.1, and 1.0%, respectively.

For experiment 2, intercepts, slopes, their respective 95% confidence interval for the Deming regression analyses, and Pearson correlation coefficients between method comparisons are presented in Table 2. The Bland-Altman plot revealed that the mean bias of the prototype against the POC_{VS} (farm gold-standard) using fresh whole blood was moderate (0.06 mmol/L; Figure 2). The limits of agreement for the prototype against the POC_{VS} cow-side were of considerable magnitude (Figure 2).

Bland-Altman plots and Deming regression analyses of the prototype and the POC_{VS} under laboratory conditions against the RA are presented in Figure 3. The use of the prototype under laboratory conditions

Table 1. Linearity experiment reporting the linear range across hypocalcemic, physiologic, and hypercalcemic ionized Ca concentrations in heparinized samples prepared from blood mixtures collected from 1 Holstein dairy cow before and after a 500-mL intravenous infusion of 23% Ca gluconate¹

Blood mixture	Method		
	Prototype ²	POC _{VS}	Reference analyzer
1	0.60 (0.58 to 0.62)	0.67	0.72
2	0.83 (0.82 to 0.84)	0.88	0.92
3	1.06 (1.05 to 1.08)	1.09	1.12
4	1.21 (1.18 to 1.22)	1.22	1.29
5	1.39 (1.37 to 1.43)	1.42	1.44
6	1.55 (1.54 to 1.58)	1.60	1.61
7	1.68 (1.67 to 1.70)	1.77	1.75
8	1.81 (1.80 to 1.82)	1.87	1.91
9	1.95 (1.95 to 1.96)	1.95	2.00

¹Measurements were performed at 37°C using 3 ion-selective electrode (ISE) instruments: an ISE Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan), a commercial point-of-care device (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA), and a laboratory reference analyzer (ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark).

²The measurements on the prototype were performed in triplicates. The mean (minimum and maximum) values are presented.

slightly decreased the variability (i.e., the difference of results were more clustered around the mean bias) in measurements of the prototype against the RA. On average, the prototype measured iCa concentrations 0.04 mmol/L higher than the RA when using heparin-balanced blood; however, the limits of agreement were still of considerable magnitude between methods. The POC_{VS} device under laboratory conditions showed a systematic bias against the RA, such that the measurements were always lower than the laboratory gold-standard. The limits of agreement between the POC_{VS} and the RA were narrower than the ones obtained with the prototype against the RA under laboratory conditions.

Finally, a clinical decision comparison was performed (objective 3), and the results obtained cow-side using fresh whole blood with the prototype and the POC_{VS} were compared against the results obtained by the RA using a heparin-balanced sample (Figure 4). The results obtained cow-side with the prototype had better

agreement with the RA than the POC_{VS} farm gold-standard comparison, such that the prototype, on average, measured iCa 0.01 mmol/L higher than the RA; however, the limits of agreement were still of considerable magnitude. When the same clinical decision comparison was performed for the POC_{VS} cow-side against the RA, the same systematically negative direction of the bias observed under laboratory conditions recurred for the POC_{VS}. The limits of agreement for the latter comparison were narrower than the ones obtained by the prototype. The negative bias observed for the POC_{VS} device seems to be independent of the type of sample (i.e., fresh non-anticoagulated or heparinized whole blood).

Calculations of Sn, Sp, and their respective 95% confidence intervals for the measurements obtained by prototype and the POC_{VS} on-farm against the RA are presented in Table 3. Using 3 different potential cut points for classification of SCH, both the POC_{VS}

Table 2. Deming regression analyses and Pearson correlation coefficients for ionized Ca measurements obtained with an ion-selective electrode (ISE) Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan), a commercial point-of-care device (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA), and a laboratory reference analyzer (ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark) in fresh non-anticoagulated whole blood (on-farm) or heparin-balanced whole blood (in the laboratory) collected from 101 periparturient Holstein cows

Reference method	Comparison method	Intercept		Slope		Pearson correlation
		Coefficient (SE)	95% CI	Coefficient (SE)	95% CI	
POC _{VS} on-farm Laboratory reference analyzer	Prototype on-farm	-0.17 (0.07)	-0.31 to -0.03	1.20 (0.06)	1.08 to 1.32	0.92
	Prototype in the laboratory	-0.16 (0.05)	-0.26 to -0.06	1.22 (0.04)	1.13 to 1.30	0.96
	POC _{VS} in the laboratory	-0.10 (0.02)	-0.14 to -0.05	1.05 (0.02)	1.01 to 1.09	0.99
	Prototype on-farm	-0.33 (0.08)	-0.49 to -0.16	1.28 (0.07)	1.14 to 1.43	0.91
	POC _{VS} on-farm	-0.14 (0.02)	-0.19 to -0.09	1.08 (0.02)	1.04 to 1.12	0.99

and the prototype performed very similarly, and high Sn (100%) and Sp ($\geq 93.5\%$) for both devices were obtained.

DISCUSSION

The objectives of our study were to assess the performance of an optimized ISE Ca-module prototype for measurement of iCa as a cow-side test and more formally evaluate the performance of a commonly used POC device. The prototype device could be of great benefit to dairy practitioners as a screening tool for SCH, as no other low-cost method is available in the medical field, and represents a significant advance to the dairy industry. Both instruments performed equally well in correctly classifying SCH and non-SCH cows

using 3 different cut point classifications, although the limits of agreement obtained by the POC_{VS} were narrower than the ones obtained by the prototype. However, we caution readers about the potential over-estimation of the Sn of both tests, as the wide range of the 95% confidence intervals indicates that not enough animals were classified as SCH in this data set. Further validation studies using a population of cows with a higher prevalence of SCH might help improve estimates of method validity.

The CLSI, an organization that provides guidelines for standardization of methods across human laboratories, has recognized the difficulty in obtaining consistency of iCa results across ISE modules between manufacturers. Moreover, no formal guidelines on limits of agreement between ISE Ca-modules have been published by CLSI yet. For instance, Uyanik et al. (2015) has shown limits of agreement for iCa between 2 blood-gas analyzers [Nova Stat Profile Critical Care Xpress (Nova Biomedical, Waltham, MA) and Siemens RapidLab 1265 (Siemens Healthcare Diagnostics Inc., Tarrytown, NY)] vary from -0.10 to 0.13 in arterial blood samples in humans. De Koninck et al. (2012) found a mean bias against their laboratory reference method as low as 0.16% and as high as 7.84% in iCa measurements among 4 portable cartridge-type analyzers used in human hospitals as patient-side devices. Comparison of 2 POC instruments to one another [VetScan i-STAT and EPOC Blood Analysis system (Epocal Inc., Ottawa, ON, Canada)] in heparinized blood samples of dogs showed a limit of agreement of ± 0.1 mmol/L between devices (West et al., 2014). It is important to note that whether a clinician decides to accept a higher degree of imprecision in a POC device depends on the type of diagnostic decision being made and whether a wrongly classified outcome might lead to a harmful consequence.

To date, no other ISE Ca-module is available as a low-cost cow-side instrument. However, several complicating factors make the application of this type of technology as a miniaturized hand-held device more difficult in the medical field. As iCa concentrations are kept to very narrow ranges in the blood of mammals, an accurate and precise tool is desirable for testing. Greater measurement accuracy in ISE modules are obtained when the sample being measured is at the same temperature of the calibration standards. That assertion is due to differences in slope calibration and ionic activity of the specimen, which vary if both the calibrators and samples are not measured under the same temperature conditions. This phenomenon has been experimentally demonstrated in a study of one of the first ISE Ca-modules under investigation for use in clinical human medicine (Arnold et al., 1968), in which measurements performed at 22 or 37°C resulted

Figure 2. Bland-Altman plot (A) and Deming regression analysis (B) displaying the performance of an ion-selective electrode (ISE) Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan) against a commercial point-of-care device (POC_{VS} ; VetScan i-STAT, Abaxis North America, Union City, CA) for measuring ionized Ca (iCa) in fresh non-anticoagulated whole blood cow-side in 101 periparturient Holstein dairy cows from 3 dairy farms in New York State.

in different millivolt readouts. As a cow-side test under field conditions, it is not feasible to standardize the temperature of the calibrators and the blood sample; therefore, some degree of variation in ionic activity is expected. In fact, our results demonstrate that a lesser degree of variability in iCa measurements were obtained for the prototype in the laboratory, in which the temperature of the standards were kept at the same temperature as the samples under evaluation. Another complicating factor is the potential change in blood viscosity, and therefore electric potential, as to whether a fresh whole-blood sample is used versus a heparinized sample. Sample viscosity has been shown to differ

in heparinized dog blood (Singh and Coulter, 1973); however, it is also possible that the measurement of a fresh non-anticoagulated whole-blood sample could more closely resemble the true iCa status of the animal, as the sample being measured is done immediately after collection without any added anticoagulants.

In our study, the POC_{VS} measured iCa concentrations consistently lower than our laboratory RA. Therefore, we caution clinicians and dairy consultants about the interchangeable use of reference intervals estimated for benchtop blood-gas analyzers against the POC_{VS} due to the negative constant bias we detected. To the best of our knowledge, only one other study has evaluated the

Figure 3. Bland-Altman plots and Deming regression analyses demonstrating the performance under laboratory conditions of ionized Ca (iCa) measurements obtained by an ion-selective electrode Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan) and a commercial point-of-care device (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA) against the laboratory reference analyzer (RA; ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark) using whole blood collected into heparin-balanced syringes in 101 periparturient Holstein dairy cows from 3 dairy farms in New York State. Graphs A and B are a comparison between the prototype and the laboratory reference analyzer. Samples measured in the prototype were kept at room temperature (approximately 22°C). Graphs C and D are a comparison between the POC_{VS} and the laboratory RA.

POC_{VS} performance for iCa measurements using bovine blood from 24 clinically healthy individuals (Peiró et al., 2010); that study did not show the same systematic negative bias as we observed, which may be due to the lower sample number or between-instrument and cartridge type variation. We are unaware of any literature that has compared the variability in iCa measurements between 2 or more POC_{VS} devices while using the same type of cartridge and batches. Moreover, the study of Peiró et al. (2010) showed that the limits of agreement varied ± 0.1 mmol/L between the portable analyzer and

their reference method; those authors concluded that the iCa measurements performed in the POC_{VS} device are reliable.

One limitation of our analyses was the comparison of a heparin-balanced sample versus a fresh non-anticoagulated whole-blood sample. Even though the time from sample collection to laboratory analysis in the benchtop blood-gas analyzer was within a narrow time frame, 2 biases could have been introduced: unknown differences in iCa between sample types and variations in the analyte concentration due to delayed analysis

Figure 4. Bland-Altman plots and Deming regression analyses demonstrating a clinical decision comparison between an ion-selective electrode Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan) and a commercial point-of-care device (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA) using results of ionized Ca (iCa) obtained cow-side (fresh non-anticoagulated whole blood) on the prototype and POC_{VS} against results obtained from a laboratory reference analyzer (RA; ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark) on whole blood collected in heparin-balanced syringes in 101 periparturient Holstein dairy cows from 3 dairy farms in New York State. Graphs A and B are a comparison between the prototype and the laboratory reference analyzer. Graphs C and D are a comparison between the POC_{VS} and the laboratory RA.

Table 3. Sensitivity (Sn), specificity (Sp), and 95% CI of an ion-selective electrode (ISE) Ca-module prototype (HORIBA Advanced Techno, Kyoto, Japan) and a commercial point-of-care device (POC_{VS}; VetScan i-STAT, Abaxis North America, Union City, CA) in classifying subclinical hypocalcemia at 3 different cut points (≤ 0.95 , 1.00, and 1.05 mmol/L) cow-side using a fresh, non-anticoagulated whole-blood sample¹

Cow-side method	≤ 0.95 mmol/L		≤ 1.00 mmol/L		≤ 1.05 mmol/L	
	% Sn (95% CI)	% Sp (95% CI)	% Sn (95% CI)	% Sp (95% CI)	% Sn (95% CI)	% Sp (95% CI)
Prototype	100 (15.8 to 100)	98 (92.8 to 99.8)	100 (39.8 to 100)	96 (89.7 to 98.9)	100 (59 to 100)	97 (90.8 to 99.3)
POC _{VS}	100 (15.8 to 100)	97 (91.3 to 99.4)	100 (39.8 to 100)	97 (91.1 to 99.4)	100 (59 to 100)	93.6 (86.5 to 97.6)

¹The true ionized Ca status was considered to be the measurement obtained by the heparin-balanced syringe analyzed in a laboratory reference analyzer (ABL-800 FLEX, Radiometer Medical, Copenhagen, Denmark).

with changes of pH. It is impractical to place a reference method such as a benchtop blood-gas analyzer at a farm for side-by-side comparisons. Therefore, to the best of our ability, a heparin-balanced syringe was used in an attempt to minimize variations in iCa concentrations that could have occurred in the delayed analyses. In human blood, heparin-balanced syringes have been shown to not alter the iCa concentrations when compared with fresh whole blood analyzed within 5 min of blood sampling (Toffaletti et al., 1991). Haverstick et al. (2009) showed an iCa mean bias of -0.01 mmol/L when human blood collected in a heparin-balanced syringe was analyzed immediately after collection and 7 h later.

Another potential limitation of our study is that, while calibrations were being made before every single blood measurement in the prototype, no QC check was performed as to verify whether the calibrations were being done correctly. Correctness during calibrations was solely based on the first author's experience. For instance, the POC_{VS} device runs a QC check before every cartridge is analyzed, and no output of the results are given if the cartridge does not pass its quality check. A QC check has not yet been fully developed for the prototype, and potential drifts in the zero potential or slope set points could have occurred during calibration of the prototype that could have contributed to some variability in iCa measurements by the prototype. Recently, Newman and Behling-Kelly (2016) discussed critical points in quality assurance and control while using POC devices in veterinary medicine. As discussed by those authors, the ideal scenario would be the inclusion of at least 2 QC levels (i.e., a normal and an abnormal level) to assure appropriate method performance. A QC check would be of extreme importance to the prototype, as it is questionable whether the average end-user will obtain the same results as the one attained in the current study. Further studies are necessary after a QC check is developed and the method starts to be commercially available.

Last, as the first author is well acquainted with the developed technology and was solely responsible for the

operation of the prototype device during all the studies presented herein, very strict calibration protocols were instituted. Readers must be aware that the prototype may yield more variable results if the prototype device is not correctly calibrated before sample measurement or if performed by more than 1 operator with different levels of technical expertise. The effect of these preanalytical variables on iCa measurements with the prototype may be lessened after development of a QC check, and further work attempting to modify the calibration procedures for improved ease of clinical use are performed (such as assessment of 1-point vs. 2-point calibration methods and a reduced requirement of the frequencies of calibrations). In addition, interference studies are warranted to estimate the potential bias in iCa measurements associated with changes in electrolytes found in some disorders of postparturient cows (e.g., clinical hypocalcemia, displaced abomasum), as cation imbalances (e.g., Na⁺, K⁺, and Mg²⁺) could potentially cause errors in iCa measurements by changing the activity of iCa in the sample or influencing electrode response time.

CONCLUSIONS

An optimized ISE Ca-module prototype was demonstrated to be a potentially valuable tool for measurement of blood iCa cow-side if SCH classification cut points can be established based on the Ca ionic form. Further testing using a population of cows with a higher prevalence of low iCa concentrations could lead to a better assessment of prototype Sn and Sp for SCH detection. Although the POC_{VS} was demonstrated to be an acceptable tool in bovine practice, we caution that it may overestimate the true incidence of SCH, considering the systematic negative bias we found in our 1 tested device. It is advisable to verify the performance of each used POC_{VS} against a reference method to ensure that correct assessment of iCa status in individual patients is interchangeable across devices. Short- and long-term imprecision, as well as studies of potential differences between- and within-cartridge

batches, should be performed to assess the validity of the POC_{VS} method for research use.

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REFERENCES

- Arnold, D. E., M. J. Stansell, and H. H. Malvin. 1968. Measurement of serum ionic calcium using a specific ion electrode. *Am. J. Clin. Pathol.* 49:627–634.
- Burrit, M. F. 1993. Electrolytes and blood gases (ionized calcium). *Anal. Chem.* 65:409–411.
- CLSI. 2013. EP09–A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed. CLSI, Wayne, PA.
- De Koninck, A. S., K. De Decker, J. Van Bocxlaer, P. Meeus, and L. Van Hoovels. 2012. Analytical performance evaluation of four cartridge-type blood gas analyzers. *Clin. Chem. Lab. Med.* 50:1083–1091.
- Forman, D. T., and L. Lorenzo. 1991. Ionized calcium: Its significance and clinical usefulness. *Ann. Clin. Lab. Sci.* 21:297–304.
- Haverstick, D. M., L. B. Brill 2nd, M. G. Scott, and D. E. Bruns. 2009. Preanalytical variables in measurement of free (ionized) calcium in lithium heparin-containing blood collection tubes. *Clin. Chim. Acta* 403:102–104.
- Lam, V., S. S. Dhaliwal, and J. C. Mamo. 2013. Adjustment of ionized calcium concentration for serum pH is not a valid marker of calcium homeostasis: implications for identifying individuals at risk of calcium metabolic disorders. *Ann. Clin. Biochem.* 50:224–229.
- Lincoln, S. D., and V. M. Lane. 1990. Serum ionized calcium concentration in clinically normal dairy cattle, and changes associated with calcium abnormalities. *J. Am. Vet. Med. Assoc.* 197:1471–1474.
- Newman, A. W., and E. Behling-Kelly. 2016. Quality assurance and quality control in point-of-care testing. *Top. Companion Anim. Med.* 31:2–10.
- Peiró, J. R., A. S. Borges, R. C. Goncalves, and L. C. Mendes. 2010. Evaluation of a portable clinical analyzer for the determination of blood gas partial pressures, electrolyte concentrations, and hematocrit in venous blood samples collected from cattle, horses, and sheep. *Am. J. Vet. Res.* 71:515–521.
- Piccione, G., V. Messina, A. Schembari, S. Casella, C. Giannetto, and D. Alberghina. 2011. Pattern of serum protein fractions in dairy cows during different stages of gestation and lactation. *J. Dairy Res.* 78:421–425.
- Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. *Vet. J.* 188:122–124.
- Singh, M., and N. A. Coulter. 1973. Rheology of blood: Effect of dilution with various dextrans. *Microvasc. Res.* 5:123–130.
- Toffaletti, J., P. Ernst, P. Hunt, and B. Abrams. 1991. Dry electrolyte-balanced heparinized syringes evaluated for determining ionized calcium and other electrolytes in whole blood. *Clin. Chem.* 37:1730–1733.
- Uyanik, M., E. Sertoglu, H. Kayadibi, S. Tapan, M. A. Serdar, C. Bilgi, and I. Kurt. 2015. Comparison of blood gas, electrolyte and metabolite results measured with two different blood gas analyzers and a core laboratory analyzer. *Scand. J. Clin. Lab. Invest.* 75:97–105.
- Wang, S., E. H. McDonnell, F. A. Sedor, and J. G. Toffaletti. 2002. pH effects on measurements of ionized calcium and ionized magnesium in blood. *Arch. Pathol. Lab. Med.* 126:947–950.
- West, E., D. Bardell, and J. M. Senior. 2014. Comparison of the EPOC and i-STAT analysers for canine blood gas and electrolyte analysis. *J. Small Anim. Pract.* 55:139–144.
- Westgard, J. O. 2008. Basic Method Validation. 3rd ed. Westgard QC Inc., Madison, WI.