J. Dairy Sci. 101:1–10
https://doi.org/10.3168/jds.2017-13779

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

R. C. Neves, T. Stokol, K. D. Bach, and J. A. A. McArt[1]
Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

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

Received September 1, 2017.
Accepted October 20, 2017.
[1]Corresponding author: jmcart@cornell.edu

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, 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