Evaluating point-of-care testing for anemia diagnosis in pigs: Blood collection location disparities, repeatability, and validity

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Abstract

The HemoCue 201 was used to compare hemoglobin (HbC) across blood sampling sites. Tail docking samples had lower HbC than both ear and mammary vein samples (P = .001). Both point-of-care and laboratory HbC testing methods showed agreement, with biases of 0.2 g/dL (ear) and -0.45 g/dL (jugular).

Keywords: swine, anemia, hemoglobin, point-of-care

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Evaluación de las pruebas en el punto de atención para el diagnóstico de anemia en cerdos: Disparidades en la ubicación de la extracción de sangre, repetibilidad, y validez

El HemoCue 201 se utilizó para comparar la hemoglobina (HbC) en los sitios de muestreo de sangre. Las muestras de corte de cola tuvieron HbC más baja que las muestras de oreja y vena mamaria (P = .001). Tanto los métodos de prueba de HbC en el punto de atención como los de laboratorio mostraron concordancia, con sesgos de 0.2 g/dL (oreja) y -0.45 g/dL (yugular).

Évaluation au point de soin pour le diagnostic de l'anémie chez les porcs: Disparités, répétabilité, et validité du site de collecte du sang

Le système HemoCue 201 a été utilisé pour comparer les taux d'hémoglobine (HbC) entre des sites de prélèvement d'échantillons de sang. Des échantillons obtenus à la suite de la caudectomie avaient un taux de HbC inférieur aux échantillons provenant de la veine de l'oreille et de la veine mammaire (P = .001). Les résultats de la méthode utilisée au point de soin et la méthode utilisée en laboratoire étaient en accords, avec un biais de 0.2 g/dL (oreille) et de -0.45 g/dL (jugulaire).

In veterinary medicine, point-of-care testing (POCT) has become increasingly common due to its rapid results and minimal blood requirement, especially in field settings. Blood hemoglobin concentration (HbC) serves as a crucial indicator of iron status in pigs, essential for growth and health. The HemoCue device has gained popularity for POCT in pig anemia investigations[1,2] despite the traditional use of laboratory hematology analyzers as the gold standard, which can be costly and impractical. Handling challenges, such as sample transport and storage, all while trying to avoid issues such as hemolysis and clotting, underscore the practical benefits of POCT in providing immediate and reliable results.

Studies evaluating the HemoCue device in pigs show conflicting results. Kutter et al[3] found agreement between HemoCue and laboratory results when testing arteriole blood, with a difference of -0.1 g/dL across measured values of 3.2 to 10.8 g/dL. Conversely, Maes et al[4] reported a slight overestimation of 0.49 g/dL by the HemoCue device compared to laboratory results when sampling from the jugular vein for laboratory testing and the ear vein for the HemoCue device. Variations in blood sampling location may have contributed to these inconsistencies. Discrepancies have been identified in human studies that employed varying anatomical locations for HbC measurement according to a review article of HemoCue validation studies.[5] Consideration of anatomical variation in swine HbC testing may be crucial for determining suitable sampling sites when using the HemoCue for HbC testing.

The ear vein is commonly used for HbC POCT in swine due to convenience and minimal invasiveness compared to the jugular vein. However, concerns exist regarding reliability of the ear vein and potential differences in HbC levels across anatomical sites, impacting critical measurement accuracy for clinical decisions. Our study compared HbC values across samples collected from ear vein, mammary vein, and tail sampling sites using a POCT device (HemoCue 201+ Hb system). Additionally, we compared POCT results with laboratory testing (Siemens Advia 2120/21201 hematology system analyzer) of samples from both ear and jugular venous sites. We also assessed the device's reliability through repeat measurements. The study aimed to determine site influence on HbC values and validate the POCT device for diagnosing pig anemia.

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Animal care and use

All procedures conducted in this study were subject to approval by the South Dakota State University (SDSU) Institutional Animal Care and Use Committee (IACUC No. 2209-051) and adhered to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (4th edition, 2020). The animals involved in this experiment were raised and managed within the sow barn at the SDSU Swine Education and Research Facility. The study took place between February 2024 and March 2024.

Materials and methods

Hemoglobin sampling and analysis

In this experiment, the single POCT device used to assess HbC was a HemoCue 201+ Hb analyzer (HemoCue America). This portable device used microcuvettes into which a small quantity (< 10 µL) of blood was loaded for analysis. The microcuvettes were analyzed with the POCT system using a photometric method at a wavelength of 570 nm. The resulting HbC was displayed and recorded within 60 seconds. The laboratory testing measurements in this study were conducted by the SDSU Animal Research and Diagnostic Laboratory using a Siemens Advia 2120/21201 hematology system (Siemens Healthcare Diagnostics), which employed the standard hemiglobincyanide test method.

Experiment 1: POCT repeatability

Repeatability of HbC measured in samples from the ear vein was determined using a total of 10 lactating sows, ranging from parity 1 to 4, and in two randomly selected 1-day-old piglets from each sow (n = 20 suckling piglets). For each subject, the ear vein was pricked once and HbC was measured three times using three separate microcuvettes from the same ear vein prick followed by immediate analysis using the POCT device.

Experiment 2: POCT location comparison testing

Thirty-eight piglets from three litters were selected for HbC testing at three different collection locations: ear vein, mammary vein, and tail. Blood samples were collected from each piglet at 1 day of age at the time of processing (ie, tail docking and iron supplementation). Ear and mammary vein blood collections were performed by pricking the respective vein using a 20-gauge needle. Blood from the tail was collected following the tail docking procedure. All samples from each location were immediately analyzed using the POCT device as previously described.

Experiment 3: POCT vs laboratory testing

Twenty-one sows, ranging from parity 1 to 4, were selected for this experiment. On day 7 of lactation, HbC measurements were taken from both the ear vein and jugular vein. Ear vein samples were collected using a 20-gauge, 2.5-cm needle and analyzed via POCT. Whole blood samples were also collected from the ear vein using s-monovette 1.3-mL, low-volume blood collection tubes containing EDTA as an anticoagulant (Thermo Fisher Scientific). Jugular vein blood was collected into 6-mL tubes containing EDTA (Becton, Dickinson and Company). Approximately 500 µL was immediately removed using a sterile syringe, with approximately 10 µL of blood placed into a microcuvette for POCT analysis. All blood tubes were transported to the SDSU Animal Research and Diagnostic Laboratory at room temperature (25°C) for HbC analysis. The mean (SD) time from collection to analysis was 3.9 (2.6) hours, and no specimens were analyzed after 12 hours.

Statistical analyses

To validate our statistical approach, we confirmed non-violation of the analysis of variance assumptions, including homogeneity of variances and normal distribution. Data are presented as mean (SD) or frequency when appropriate. An analysis of variance using Proc MIXED in SAS (version 9.4, SAS Institute Inc) was conducted to compare HbC in blood obtained from different locations (ear vein, mammary vein, and tail) and differences between the two testing methods (POCT vs laboratory testing). Bland-Altman analysis was conducted to calculate bias and limits of agreement (LOA) to assess agreement between methods. Anemia, defined as < 10 g/dL, was determined for each sample.[6,7] The prevalence of anemic and nonanemic animals was compared using a Chi-square test for frequency. Differences with $P < .05$ were considered statistically significant.

Results

POCT repeatability

Hemoglobin concentration from the three consecutive samples taken from the ear vein among the 38 pigs resulted in mean HbC values of 9.31 (1.4), 9.30 (1.2), and 9.33 (1.2) g/dL for samples 1, 2, and 3 across all animals, respectively. The average coefficient of variation determined between means within animals was 3.65%. When classifying each animal as anemic (< 10 g/dL) or nonanemic (≥ 10 g/dL) using each of the three samples taken, 3 of 38 pigs did not have the same classification across the 3 samples taken.

POCT location comparison

Ear and mammary vein HbC values were not different from one another ($P = .64$), while the ear and mammary vein HbC values were both higher compared to the tail HbC ($P < .001$; Table 1). Anemia prevalence varied between locations, with the highest prevalence occurring when using HbC values from the tail (92.1%) followed by the ear vein (55.3%), and the lowest prevalence occurring when using the mammary vein (39.5%) ($X^2 = .001$).

POCT vs laboratory testing

There was no difference ($P = .99$) observed in HbC values between ear vein samples analyzed with POCT and those analyzed with laboratory testing (Table 2). Similarly, no difference ($P = .91$) was observed in HbC values between jugular vein samples analyzed with the POCT and laboratory testing. When comparing HbC values between ear vein samples analyzed with POCT and jugular vein samples analyzed using laboratory testing, no difference was observed ($P = .98$). Similarly, there was no difference ($P = .89$) between jugular vein samples analyzed with POCT and ear vein samples analyzed with laboratory testing. Ear vein samples analyzed with POCT exhibited a bias of 0.2 g/dL with LOA of -1.1 to 1.5 compared to laboratory testing HbC values. For jugular blood, HbC values using POCT showed a bias of -0.45 g/dL with LOA of -1.4 to 0.53 compared to laboratory testing.

Table 1: Comparative analysis of hemoglobin measurement using a point-of-care testing method* to assess different blood draw sites in 1-day-old piglets

Blood draw site	Samples, No.	Anemia, %†	Mean HbC, g/dL
Ear vein	38	55.3[a]	9.8[a]
Mammary vein	38	39.5[b]	10.1[a]
Tail dock	38	92.1[c]	7.2[b]
SEM	NA	NA	2.5
P	NA	NA	< .001
X^2	NA	< .001	NA

* HemoCue 201 Hb analyzer.

† Anemia was defined as < 10 g/dL blood hemoglobin concentration.

a,b,c Different superscripts with the same column indicate differences at *P* < .05.

HbC = hemoglobin concentration; NA = not applicable.

Table 2: Comparison between POCT* and laboratory testing† in sows using blood samples taken from two different sites

		Mean HbC, g/dL			
Location	No. of samples	POCT	Laboratory testing	SEM, g/dL	*P*
Ear	21	10.8	10.7	0.3	.99
Jugular	21	10.4	10.7	0.3	.91
SEM		0.3	0.3	NA	NA
P		.73	.99	NA	NA

* HemoCue 201 Hb analyzer.

† Siemens Advia 2120/21201 hematology system.

POCT = point-of-care testing; HbC = hemoglobin concentration; NA = not applicable.

Discussion

The POCT method used in this study provides a rapid and cost-effective solution for on-farm HbC assessment. Consistent mean HbC values were observed with acceptable repeatability from consecutive ear vein samples, supported by low variability within each animal. It is worth noting that a few pigs near the 10 g/dL HbC cutoff showed variability across repeat samples, impacting diagnostic consistency for anemia.

Differences between tail sampling and ear and mammary vein sampling may be due to tail docking blood being a mix of venous and arteriolar blood, possibly diluted by tissue damage as well. Reference HbC values in pigs have been established based on venous blood. Therefore, venous blood is recommended for diagnosing anemia using HbC cutoff values that have been previously defined. Additionally, it was frequently observed during sample collections that some piglets yielded insufficient blood from the tail docking site, posing challenges if duplicate samples were needed. While sampling at the time of tail docking is convenient and can be performed while handling the pig, its limitations in terms of blood volume were evident. Consequently, the ear and mammary veins were considered more reliable for testing HbC in newborn piglets using the POCT device.

Differences among blood collection sites highlight the importance of considering anatomical location when interpreting HbC measurements, which can influence the determination of anemia prevalence. While no significant differences in HbC levels were found between ear and mammary vein samples, anemia prevalence was higher when using ear vein samples versus mammary vein samples. This raises concerns about accuracy and the potential need for site-specific adjustments, particularly when using an anemia cutoff < 10 g/dL. Pigs categorized differently for anemia based on location were those pigs that were very close to the anemic HbC cutoff value, similar to observations with repeat samples. Based on these findings, consistency in blood collection site is crucial for monitoring HbC over time and tracking recovery post treatment in pig herds.

Diagnosis of clinical anemia should consider additional symptoms such as pale skin, labored breathing, lethargy, and inactivity. These signs may provide crucial supplementary information to confirm or challenge anemia diagnosis, particularly when HbC values are near the anemia cutoff point. Nonetheless, site-specific variations in HbC affected a small percentage of pigs for anemia diagnosis across ear, mammary, and jugular vein sampling sites in this study. Based on these findings, these are suitable blood sampling sites for HbC analysis using POCT.

When comparing these findings to previous research, human studies have indicated the HemoCue device's accuracy compared to standard laboratory tests. Differences in blood sampling location have been investigated, revealing that the site from which blood is drawn can have a small but statistically significant impact on both the mean and variability of HbC measurements. Specifically, higher HbC in capillary samples have been noted compared to venous samples.[8,9] Other studies have found acceptable accuracy when arterial and venous blood samples were assessed POCT compared to laboratory testing.[10,11] The current study found consistent measurements regardless of the specific venous site used. However, HbC from blood obtained during tail docking, which may have included venous blood, arterial blood, and tissue fluids, differed in HbC from the venous samples.

Overall, this study demonstrates that the HemoCue is a promising POCT device for measuring HbC in swine, suitable for research and field settings. Hemoglobin concentration measurement in pigs is currently infrequent, likely due to the time required for submission and cost, potentially resulting in a lack of pig anemia diagnosis. The HemoCue offers rapid and reliable results, potentially improving on-farm HbC assessment in pigs, benefiting both commercial and research applications.

Implications

Under the conditions of this study:

- Collection site HbC variations affect anemia (< 10 g/dL) diagnosis.
- Use of blood collected during tail docking is not recommended for HbC analysis.
- HemoCue reliably measures ear, jugular, and mammary vein HbC for anemia screening.

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Conflict of interest

None reported.

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