Received: 30 October 2019 | Revised: 6 February 2020 | Accepted: 7 February 2020

DOI: 10.1111/ejh.13397

ORIGINAL ARTICLE

European Journal of Haematology | WILEY

Validity of plasma collection cards for ferritin assessment—A proof-of-concept study

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Funding information
Nebraska Beef Council

Abstract

Objectives: Iron depletion is common around the world and among certain risk groups in developed countries. The overall purpose was to test the suitability of a novel plasma collection card for minimally invasive iron status assessment.

Methods: Twenty participants (10 f/10 m) participated in this cross-sectional study. Ferritin and hemoglobin were measured from blood collected from a forearm vein, serving as reference method. Blood was also collected from the fingertip using the Noviplex™ Plasma Prep Card as well as capillary collection tubes.

Results: There was substantial concordance between ferritin measured from samples collected via Noviplex™ and venous ferritin (concordance correlation coefficient (CCC) = 0.96) with a mean bias of −0.8 ng/mL. Storing Noviplex™ cards at room temperature for 2 weeks resulted in slightly lower but good concordance when compared to venous ferritin (CCC = 0.95). Capillary hemoglobin (CCC = 0.42) and hematocrit (CCC = 0.25) were in poor agreement with venous data.

Conclusions: Noviplex™ cards offer a suitable alternative for a minimally invasive ferritin screening in the field when compared to capillary collection tubes. Despite overall substantial concordance with the reference method, findings indicative of iron status abnormalities should be confirmed in venous samples.

KEYWORDS

capillary blood, ferritin, hemoglobin, iron depletion, iron status, plasma, stability

1 | INTRODUCTION

Iron deficiency is one of the most common nutrient deficiencies worldwide.[1] Although the overall prevalence of iron deficiency anemia (IDA) is low in the United States and other developed countries,[2] certain subgroups are at increased risk for iron status disorders. One group for whom iron is a particularly critical micronutrient are athletes and recreational exercisers,[3] who experience increased iron losses through various mechanisms, including excessive sweating, gastrointestinal bleeding, hemolysis, and exercise-induced inflammation.[4-6] As a result, athletes are almost three times as likely to be iron depleted when compared to non-athletes,[7] a risk that is even further exacerbated in certain groups, such as female adolescent athletes, among whom as much as 58% may suffer from iron depletion.[7,8] Iron depletion, even in the absence of anemia, can result in suboptimal athletic performance,[9-11] which can be reversed by means

of iron supplementation or dietary intervention.[12-16] Considering the wide-spread prevalence of iron status abnormalities among athletes and its detrimental effects on performance, along with the potential dangerous side effects of unmonitored supplementation,[6] regular screening and monitoring of iron status are common practice among athletes.[17] The most common screening measure of iron status is plasma ferritin, which is reflective of iron storage, along with hemoglobin and hematocrit reflecting functional iron in red blood cells.[11]

Routinely, these markers are assessed from blood samples obtained via venous blood sampling. However, venous sampling is invasive, costly, demanding in terms of equipment and requires highly trained and certified personnel. When compared to other forms of sample collection, it is more likely to result in adverse events for subjects and healthcare workers and involves greater sampling volumes.[18] Further, phlebotomy-related issues are regarded the primary source of errors occurring in the pre-analytical phase.[19] An alternative sampling method involves the collection of capillary blood, which is considered more applicable in field settings[20] and has been used for the assessment of iron status markers in various vulnerable populations.[21-23] In one of the few studies on the validity of capillary iron markers, capillary measurements of ferritin were found to be ~3%-5% higher and more variable when compared to venous blood, although the authors concluded that these issues were outweighed by the benefits of using capillary over venous blood collection.[24]

Despite this favorable cost-benefit profile, traditional capillary sampling faces challenges similar to venous sampling, such as the need to separate plasma from red blood cells, refrigeration, and transport under biosafety guidelines. Due to these limitations, numerous attempts have been made to collect blood in the field for screening purposes using methods that do not require laboratory-intensive sample preparation and handling.[20] The most promising approach involves blood spots, which have been used by various groups to measure ferritin.[18,25] These blood spots, which are typically collected on filter paper and can be stored without freezing, reduce burden and cost for sample transport and storage. However, blood spots collected on filter paper require precise knowledge of the blood volume and extensive sample processing involving incubation with cellulase prior to analysis.[18,25] Furthermore, the complete separation of plasma from red blood cells is critical for valid assessments of ferritin, as ferritin is present both in plasma and in red blood cells, albeit at much higher concentrations in the latter, which bears the risk of masking the presence of iron depletion or deficiency when cellular ferritin leaks into plasma or whole blood is analyzed.[20]

These challenges can be overcome using a novel plasma collection card, the Noviplex™ Plasma Prep Card (Novilytic, West Lafayette, IN). These cards consist of a precut absorbent collection disk underneath a membrane filter. Blood can be collected directly onto the card test area and exact aliquots of plasma are drawn through the filter by capillary action, reducing the likelihood of hemolysis and removing the need for equipment such as pipettes and centrifuges. Given these benefits over more traditional sampling methodologies, our overall goal was to test the suitability of these cards for minimally invasive assessment of iron status biomarkers. The purpose of the present proof-of-concept study was to assess the validity of ferritin concentrations in plasma samples collected using the Noviplex™ system in comparison with plasma samples obtained via venous blood collection, the clinical gold standard. To control for potential differences in collection sites, we further assessed iron status measures in traditionally collected capillary samples.

Novelty Statement

- We tested a novel methodology for plasma collection with regard to its suitability for minimally invasive ferritin assessment.
- Our central finding is that Noviplex™ PLasma Prep Cards provide acceptable concordance with the reference method of venous sampling, even when cards are stored for a prolonged time prior to analysis.
- Noviplex™ prep cards are suitable for minimally invasive sample iron status screening, particularly in settings outside of the laboratory.

2 | METHODS

The present proof-of-concept study was conducted as a cross-sectional comparison of capillary and venous markers of iron status in a convenience sample of adults (Figure 1). All procedures were approved in advance by our university's Institutional Review Board. All participants provided written consent prior to data and blood sample collection.

2.1 | Participants

Participants were recruited locally via word by mouth and fliers. Volunteers were included if they were between 19 and 65 years of age and non-pregnant. Participants were excluded if they had a history of any disease that affects red blood cell morphology or felt unable to complete venous and/or capillary blood collection. Demographic data and information pertaining to inclusion and exclusion criteria were collected by self-report using a short survey. Upon inclusion into the study, height and weight were collected using a scale and stadiometer (Seca, Hamburg, Germany).

2.2 | Sample collection

Blood samples were obtained by a trained phlebotomist via venipuncture and capillary collection. Venous blood (6 mL) was collected

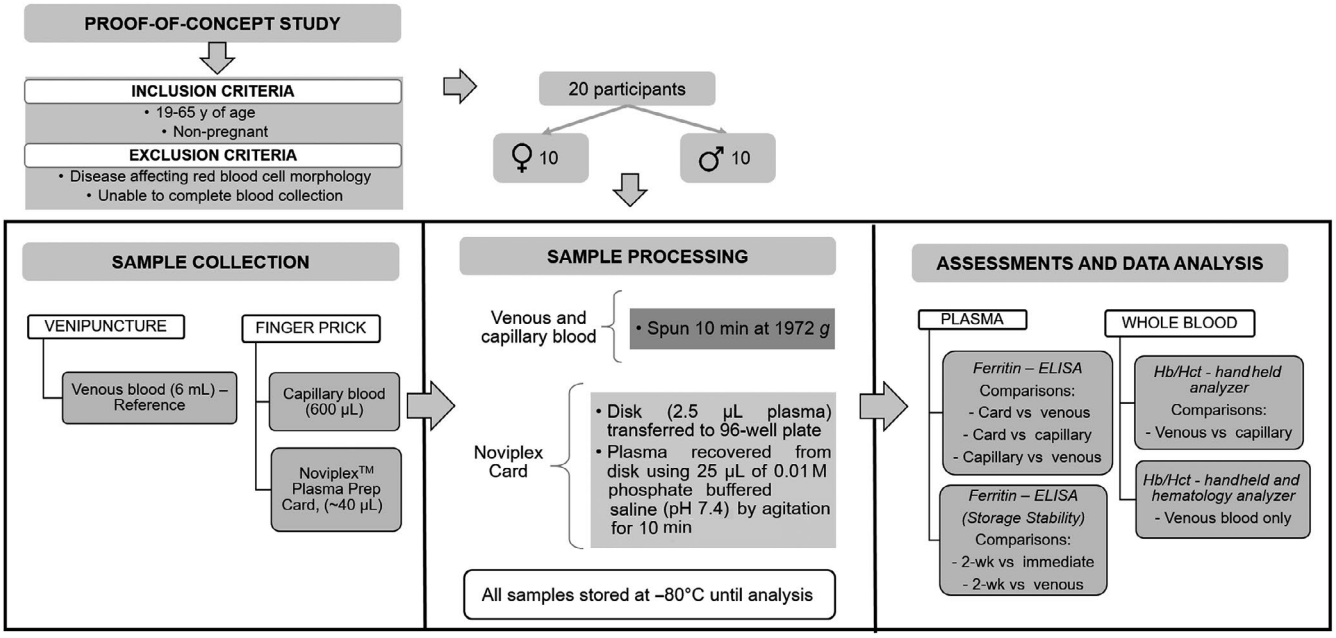

FIGURE 1 Schematic depiction of the study protocol

from an antecubital vein into EDTA-coated tubes (BD Vacutainer®). Capillary blood (600 µL) was collected via finger prick using a disposable lancet (SurgiLance™, 2.3 mm depth). The first droplet of blood was wiped away, and gentle pressure was applied to collect blood into an EDTA-coated capillary tube (Sarstedt Microvette) and directly onto the test area of a total of two Noviplex™ Plasma Prep Cards (Novilytic, North Webster, IN). For the assessment of ferritin, we used Noviplex™ UNO cards, which collect 2.5 µL of plasma onto one collection disk. Noviplex™ Plasma Prep Cards have a control indicator which signals when sufficient volume of sample has been applied. After 3 minutes, the top layer of the card containing the filter used to separate plasma from blood cells was removed, and the bottom layer containing the absorbent collection disk was allowed to dry for 15 minutes prior to further analysis. In order to assess the stability of ferritin using Noviplex™ cards, one card was processed immediately (immediate analysis), and an additional card was stored at room temperature for 14 days prior to further processing (delayed analysis).

2.3 | Sample processing

Venous blood samples as well as capillary tubes were centrifuged for 10 minutes at 1972 *g* to separate plasma and red blood cells. After centrifugation, plasma was transferred into microcentrifuge tubes and stored at −80°C until the time of analysis. For Noviplex™ cards, plasma was recovered from the collection disk using 25 µL of phosphate-buffered saline (pH 7.4) by agitation for 10 minutes using a Gyrotory shaker (New Brunswick Scientific, Edison, NJ). The recovered plasma was stored at −80°C until the time of analysis. Concentrations were subsequently corrected for the volume difference in collection and recovery.

2.4 | Assessments

Plasma concentrations of ferritin were determined using commercially available ELISA kits (Ferritin: #S-22; Ramco Laboratories, Inc, Houston, TX). ELISAs were performed by the same laboratory technician as per manufacturer instructions. Venous and capillary samples collected via capillary tubes were analyzed in duplicate and data are reported as the mean of the duplicate analysis. Recovered plasma from Noviplex™ Plasma Prep Cards was analyzed in singlet. The limit of detection was 0.59 ng/mL and assay precision in our laboratory was <8.0% for duplicate samples. For one participant, ferritin data are missing for both immediate and delayed analyses of the Plasma Prep Card because assay results fell below the limit of detection. For another participant, ferritin data are missing only for delayed analysis due to a technical error. For venous and capillary blood, we further collected whole-blood measurements of hemoglobin (Hb) and hematocrit (Hct), which were taken at the time of draw using a handheld AimStrip® Hb Meter (Germaine™ Laboratories, San Antonio, TX). As an additional reference, Hb and Hct were also measured using a hematology analyzer (QBC Autoread Plus, QBC, Port Matilda, PA), although this measurement was conducted on venous samples only.

2.5 | Data analysis and statistics

To assess the validity of Noviplex™ Plasma Prep Cards for ferritin, we compared ferritin measured from samples collected with cards to ferritin measured from samples collected via venous blood sampling, which served as the reference method. To account for differences in collection sites (capillary vs. venous) and collection techniques (cards vs. tubes), we further compared ferritin data between samples collected via capillary

tubes and venous collection and between samples collected via cards and capillary tubes. We also compared whole-blood-based markers of iron status (hemoglobin and hematocrit) from capillary and venous samples using a handheld analyzer. To account for possible errors of our handheld hematology device, we lastly compared hemoglobin and hematocrit data collected using the handheld analyzer with data assessed with our laboratory-based hematology analyzer (venous samples only).

Statistical analyses were conducted using R version 3.6.1 (The R Foundation for Statistical Computing, 2019). Precision and accuracy were determined using the concordance correlation coefficient (CCC).[26] Concordance was considered perfect for CCC ≥ 0.99, substantial for 0.95 ≤ CCC <0.99, moderate for 0.90 ≤ CCC <0.95, and poor for CCC < 0.90.[27] In addition, Bland-Altman analyses were performed to determine mean bias as well as the upper and lower 95% limits of agreement (LOA) between two methods, defined as mean difference ± 1.96 times the standard deviation.[28] To assess the clinical relevance, differences between methods were compared with a recommended desirable specification for allowable total error of 16.9%, as recommended for ferritin.[29]

3 | RESULTS

3.1 | Participants

Twenty volunteers (10 male and 10 female) between 19 and 56 years of age participated in this analysis (Table 1). Average participant age and body mass index of our convenience sample were 28.5 ± 8.3 years and 26.5 ± 5.3 kg/m^2, respectively. The majority of participants were white/Caucasian/European (65%), and the remaining participants were Asian (10%), Hispanic (10%), Black/African American (5%), and mixed race (10%). One male participant had IDA (venous hemoglobin < 13 g/dL and ferritin < 12 ng/mL). Three female participants were considered iron deplete (venous ferritin between 12-25 ng/mL). Iron overload, defined as venous ferritin > 200 ng/mL, was evident in one male participant. All remaining participants fell within the normal ranges for hemoglobin and ferritin. Venous hemoglobin concentrations were significantly higher in male than in female participants (P = .028), as was venous hematocrit (P = .019). Venous ferritin (P = .52) was not significantly different between male and female participants.

3.2 | Validity of ferritin measurements

Overall, there was substantial concordance (CCC = 0.96) between plasma ferritin measured with Noviplex™ cards and the reference standard, ferritin measured from venous blood samples (Figure 2). Bland-Altman analysis revealed no systematic bias (mean difference = −0.8 ng/mL) with upper and lower limits of agreements ranging from −23.0 to 21.3 ng/mL. The total error between plasma ferritin measured with Noviplex™ cards and venous ferritin exceeded the recommended desirable range of 16.9% in 8 samples (40%). Because the accurate assessment of ferritin near the thresholds for iron depletion (25 ng/mL) and iron deficiency (12 ng/mL) is particularly critical, a separate analysis was conducted utilizing only data from individuals with a venous ferritin < 75 ng/mL (data not shown). While the mean difference remained almost unchanged (0.2 ng/mL), the upper and lower limits of agreement narrowed (−9.3 to + 9.6 ng/mL).

In order to account for differences in sampling sites, we compared plasma ferritin measured with Noviplex™ cards to ferritin measured from capillary blood samples, which showed moderate concordance between the two methods (CCC = 0.91) with a mean bias of −10.7 ng/mL and limits of agreement ranging from −40.5 to 19.0 ng/mL. A total of 8 samples (40%) were outside of the recommended desirable range of 16.9% for the total error. The concordance between capillary and venous samples was substantial (CCC = 0.96) with a mean bias of 9.9 ng/mL and limits of agreement ranging from −8.2 to 27.9 ng/mL. The total error between plasma ferritin measured from capillary and venous samples exceeded 16.9% in 4 samples (20%). When comparing agreement between duplicate ferritin measures (Figure S1), concordance was considered substantial for venous (CCC = 0.97) and perfect for capillary (CCC > 0.99) samples.

3.3 | Storage stability

The impact of storing the Noviplex™ card at room temperature for 2 weeks prior to analysis on capillary ferritin is shown in Figure 3. Overall, there was moderate concordance (CCC = 0.94) between cards processed immediately and cards stored for 2 weeks. The mean bias was 10.5 ng/mL, with limits of agreement ranging from −15.4 to 36.5 ng/mL. Concordance between delayed analysis and venous ferritin was good (CCC = 0.95) with a mean bias of −6.8 ng/mL and limits of agreement ranging from −31.1 to 17.5 ng/mL.

3.4 | Impact of collection site on other hematological parameters

When assessing the validity of other hematological parameters measured from capillary blood, there was poor concordance for

TABLE 1 Basic demographic and hematological parameters of our convenience sample (n = 20)

	Age (y)	BMI (kg/m^2)	Hemoglobin (g/dL)	Hematocrit (%)	Ferritin (ng/mL)
Men	25.6 ± 5.8	26.6 ± 4.1	14.7 ± 1.2	45.7 ± 3.5	85.6 ± 98.2
Women	31.4 ± 9.8	26.4 ± 6.5	13.7 ± 0.4	42.5 ± 1.6	62.9 ± 47.6

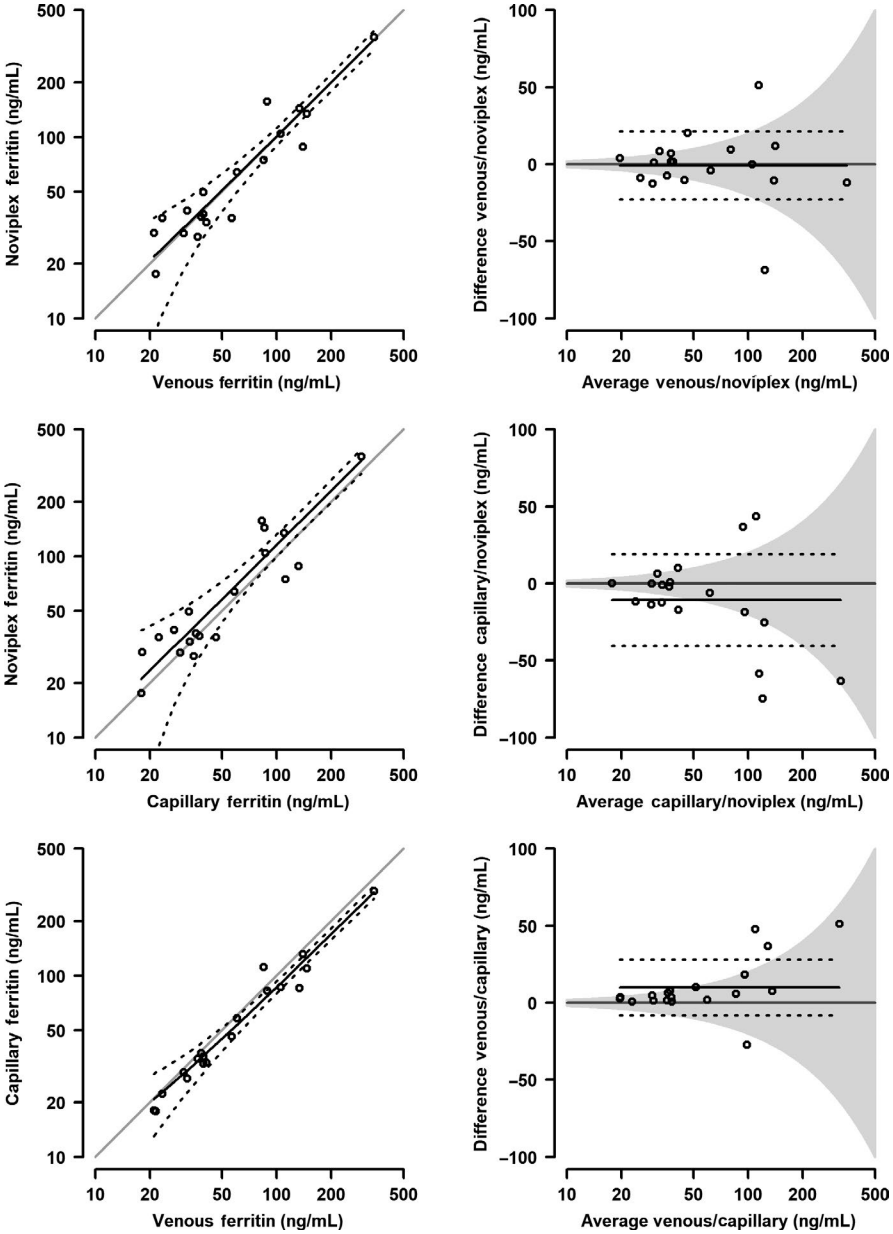

FIGURE 2 Left column: Agreement between plasma ferritin measured from blood samples collected from a forearm vein, which served as reference standard (venous ferritin), from samples collected using the Noviplex™ Plasma Prep Cards (Noviplex ferritin), and from samples collected via capillary collection into a tube (capillary collection). The gray lines denote the lines of identity. The solid black line depicts the linear regression, and dashed lines indicate the 95% confidence intervals. Right column: Bland-Altman analyses of plasma ferritin, Noviplex ferritin, and capillary ferritin. The dark gray line denotes the line of identity. The solid black line depicts the mean bias, and dashed lines indicate the 95% limits of agreement. The gray shaded area indicates a desirable allowable total error of 16.9% as suggested previously[29]

hemoglobin (CCC = 0.42) and hematocrit (CCC = 0.25) when compared to values obtained from venous blood measured with our laboratory-based hematology analyzer. Mean biases were 0.25 g/dL (hemoglobin) and 2.86% (hematocrit) and limits of agreement ranged between −0.77 and 1.27 g/dL (hemoglobin) and −0.4 and 6.2% (hematocrit). Concordance was similarly poor when comparing the capillary hemoglobin (CCC = 0.44) and hematocrit (CCC = 0.45) with values obtained from venous blood measured with the same handheld analyzer. Mean biases were 0.37 g/dL (hemoglobin) and 1.0% (hematocrit) and limits of agreement ranged between −0.71 and 1.46 g/dL (hemoglobin) and −2.1 and 4.1% (hematocrit). Concordance between the handheld analyzer and the hematology analyzer using venous samples was improved but remained poor for hemoglobin (CCC = 0.87) and hematocrit (CCC = 0.76), as were mean biases (hemoglobin: −0.1 g/dL, hematocrit: 1.9%) and limits of agreement (hemoglobin: −0.7 to 0.4 g/dL, hematocrit: 0.5 to 3.3%), the latter indicating that the handheld device significantly underestimated hematocrit.

4 | DISCUSSION

The overall goal of this proof-of-concept study was to evaluate whether Noviplex™ Plasma Prep Cards, a novel blood collection system, are suitable for minimally invasive screening of iron status. Our primary finding was that plasma concentrations of ferritin, when assessed from samples collected with the Noviplex™ cards, were in overall agreement with plasma ferritin assessed from samples obtained via the gold standard method of venous blood collection, as demonstrated by a substantial concordance (CCC = 0.96) and between the two methods. While there was almost an identical concordance with the reference standard for ferritin measured from

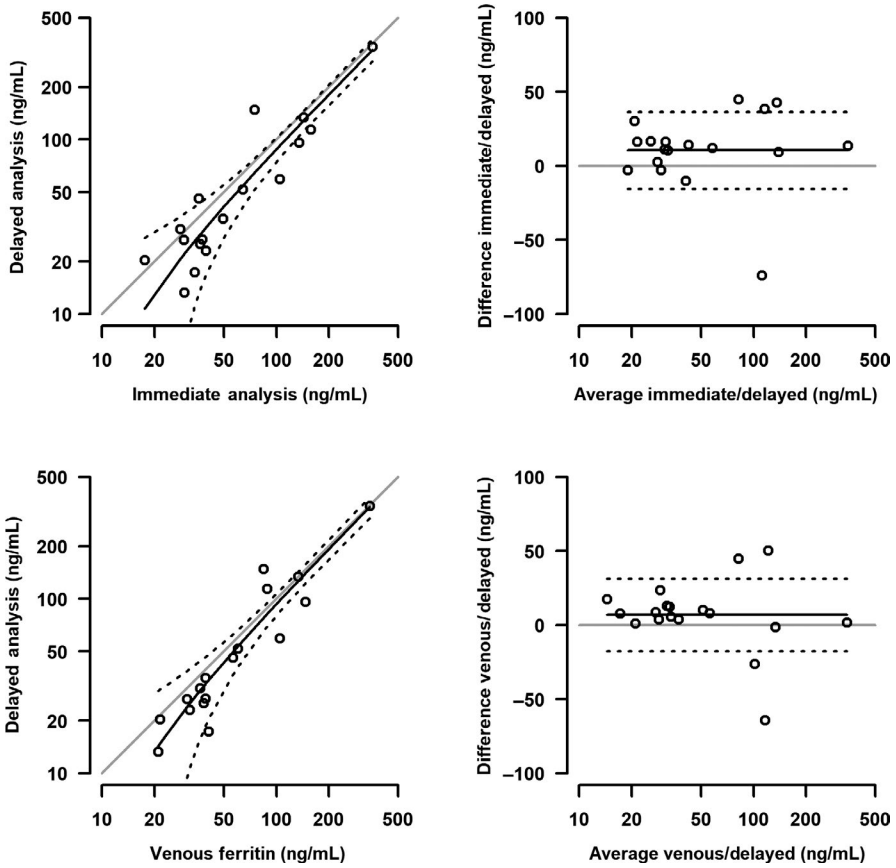

FIGURE 3 Left column: Agreement between ferritin measured from blood samples collected using the Noviplex™ cards analyzed after storing the cards for 2 wk at room temperature (delayed analysis) with samples collected using the Noviplex™ cards which were processed immediately (immediate analysis) and with ferritin measured from ferritin collected from a forearm vein (venous ferritin). The gray line denotes the line of identity. The solid black line depicts the linear regression, and dashed lines indicate the 95% confidence intervals. Right column: Bland-Altman analyses of delayed analysis, immediate analysis, and venous ferritin. The gray line denotes the line of identity. The solid black line depicts the mean bias, and dashed lines indicate the 95% limits of agreement

capillary samples (CCC = 0.96), Noviplex™ cards showed virtually no mean bias (−0.8 ng/mL), and classic capillary collection demonstrated a mean bias of 9.9 ng/mL when compared with venous collection. This finding is in agreement with previous findings of slightly elevated ferritin concentrations in capillary samples.[24] Since hemolysis is a common risk with capillary sample collection[30] and can lead to the leaking of ferritin from within red blood cells, where concentrations are much higher than in plasma,[20] it is possible that the slight elevation in ferritin in traditionally collected capillary samples is the result of contamination with cell-derived ferritin. With regard to sampling sites, it is further important to note that hemoglobin and hematocrit values obtained from capillary samples showed only poor agreement with those from venous samples. While the validity of capillary hemoglobin and hematocrit was not the primary purpose of our study, our findings indicate that the collection of samples with Noviplex™ card provide certain benefits over traditional capillary blood collection into tubes.

The benefits of the Noviplex™ cards, namely the use of a smaller sample volume (~40 µL vs. 400 µL) and the eliminated need for pipetting or other equipment (eg, centrifuge, refrigeration), mirror those reported for dried blood spots. A recent study by Siart et al (2019) assessed the use of dried blood spots for the assessment of inflammatory markers in a group of 12 male individuals. And although their analysis included a large number of analytes, correlation coefficients between venous concentrations and those obtained from dried blood spots collected from capillary blood were smaller for all 92 analytes (< 0.92[31]) when compared to our results (r = .96).

Another important finding of our study was that samples collected using the Noviplex™ were fairly stable over two weeks of storage at room temperature. Ferritin measured from cards stored at room temperature for 2 weeks demonstrated good concordance when compared both to our reference method of venous samples processed immediately (CCC = 0.95) and to Noviplex™ cards analyzed immediately (CCC = 0.94). These findings are important to consider for the handling of samples, as there is no need for refrigeration. Using the Noviplex™ system, samples can be collected on-site and subsequently sent to the laboratory for analysis, a procedure which is highly advantageous for in-field assessments. When combined with the ease of sample collection, the substantially smaller sampling volume, and a lower risk of bruising and infections, this flexibility opens many new avenues for the iron status assessment in many different scenarios, such as athletes travelling to remote training and competition sites. Despite the economic burden of iron status assessments in elite athletes,[17] wide-spread iron status screening to support an athlete's health and performance is strongly advocated in the athletic community.[32] Even though there was overall good concordance for cards stored at room temperature for 2 weeks, ferritin concentrations were slightly reduced, as indicated by mean differences of 6.8 ng/mL (when compared to venous samples) and 10.5 ng/mL (when compared to card analyzed immediately), a difference which should be accounted for when making clinical decisions based solely on samples stored for a prolonged period prior to analysis.

Despite the substantial concordance and the virtually non-existent mean difference between ferritin measured from samples

collected via the Noviplex™ system and samples collected via venous sampling, it should be noted that 40% of the samples fell outside of the previously recommended desirable specification for allowable total error of 16.9%.[29] It is worth noting that the desirable range for the total error was exceeded primarily in samples with ferritin concentrations toward the lower end of the concentration range, where the allowable error is rather narrow. For samples in the range of the cutoffs for IDA (12 ng/mL) and iron depletion (20-35 ng/mL),[33] a total error of 16.9% equates to 2 ng/mL and 3-6 ng/mL, respectively. If the Noviplex™ system is only used as a screening tool, implications of false-positive findings (ie, wrongly identifying a person as iron depleted) are minor as long as confirmation analyses are conducted via venous sampling. On the other hand, false-negative findings (ie, failing to identify a person as iron depleted) may result in failure to provide dietary intervention or supplementation to iron-depleted individuals. Considering these implications, we propose that individuals whose ferritin measured from plasma collected via the Noviplex™ system is <50 ng/mL should undergo follow-up assessments. This procedure would ensure that both individuals with iron deficiency (ferritin < 12 ng/mL) and those with a high likelihood of iron depletion (<20-35 ng/mL) would be identified for follow-up analyses.

It should be noted some variation between ferritin measured from samples collected with the Noviplex™ plasma prep cards and our reference might be due to analytical error, which we quantified by comparing duplicate measures for ferritin from venous and capillary samples. While there was almost perfect concordance between duplicate measures for capillary ferritin (CCC > 0.99), the concordance for duplicate measures of venous ferritin from the same sample (CCC = 0.97) was only slightly higher when compared to the concordance of venous and Noviplex ferritin (CCC = 0.96). Due to the small sample volume of the Noviplex™ plasma prep cards of ~5 µL plasma, we were unable to conduct to measurements in duplicate, an approach which future studies may want to pursue in order to minimize the analytical error.

The primary limitations of our study include its relatively small sample size of 20 participants, who were recruited from a convenience sample rather than conducting the validation study in athletes or other groups at risk for iron status abnormalities. However, given the high prevalence of iron depletion and IDA in these groups, the wider range of ferritin concentrations from iron deficiency to overload allowed us to test the validity of the Noviplex™ cards across the entire ferritin range. Building on the findings of this proof-of-concept study, the next step requires a full validation of the collection method for ferritin and possibly other biomarkers of iron status in a larger sample.

5 | CONCLUSIONS

The findings of this proof-of-concept study suggest that the Noviplex™ cards offer a suitable alternative for the evaluation of iron status as determined by plasma ferritin when compared to capillary blood collection into collection tubes requiring sample processing. The benefits of the Noviplex™ cards, which include a small sample volume, easy sample processing and handling, the absence of expensive equipment (eg, centrifuge and refrigeration), and relative stability over prolonged storage at room temperature make the system suited for minimally invasive iron status screenings in the field. Despite substantial concordance with venous blood collection, abnormal findings indicative of iron status abnormalities should be confirmed in venous samples prior to the initiation of dietary or pharmacological interventions.

ACKNOWLEDGEMENTS

This work was in part supported by a grant from the Nebraska Beef Council. The Noviplex™ Plasma Prep Cards were provided without charge by Novilytic, West Lafayette, IN.

CONFLICT OF INTEREST

Jiri Adamec is co-founder of Novilytic but was not involved in any aspect of the experimental design and data interpretation. Karsten Koehler, Eileen Marks-Nelson, Camila Braga, and Safiya Beckford have nothing to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Koehler K, Marks-Nelson E, Braga CP, Beckford S, Adamec J. Validity of plasma collection cards for ferritin assessment—A proof-of-concept study. *Eur J Haematol*. 2020;104:554–561. https://doi.org/10.1111/ejh.13397