Ferritin concentrations in dried serum spots from capillary and venous blood in children in Sri Lanka: a validation study[1–3]

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ABSTRACT

Background: Assessing iron status continues to be challenging in field situations. Spot methods developed for analyzing ferritin from serum or plasma samples that are spotted and dried on filter paper have been shown to provide reliable and accurate iron-status assessments. However, the spot methods are based on samples from venous serum or plasma and have not been evaluated in field settings.

Objective: We evaluated the validity of analyzing ferritin to assess iron status by using venous and capillary dried-serum-spot (DSS) samples by the spot method compared with using serum ferritin by the traditional method in a field setting.

Design: Venous and capillary blood was obtained from healthy schoolchildren (n = 100; $\bar{x}$ ± SD age: 8.9 ± 0.3 y) in Colombo, Sri Lanka. To prepare DSS samples, we aliquoted precisely 20 μL serum per spot on filter paper, air-dried the spots, and placed them in airtight plastic bags until analysis by the spot ferritin method with the use of cellulase from *Trichoderma reesei* at 2 wk after collection. Venous serum (100 μL) was frozen until ferritin determination by traditional radioimmunoassay.

Results: Venous and capillary DSS ferritin values correlated strongly with traditional serum ferritin values (r = 0.88 and 0.86, respectively; P = 0.0001). The geometric means (±1 SD) for venous and capillary DSS ferritin and traditional ferritin were 26.9 (15.3–47.4), 33.9 (20.9–54.8), and 33.1 (18.6–58.8) μg/L, respectively, and were not significantly different. Venous and capillary DSS methods on average (±SD) yielded ferritin values that were 5.8 ± 10.1 μg/L lower and 0.1 ± 9.4 μg/L higher, respectively, than serum ferritin values obtained with the traditional method.

Conclusions: Capillary and venous DSS methods for analyzing ferritin provide accurate tools for assessing iron status. Furthermore, capillary DSS ferritin is a practical means of detecting iron deficiency in field settings. *Am J Clin Nutr* 2002;75:289–94.

KEY WORDS Iron-status assessment, ferritin, dried serum spots, capillary blood and venous blood, filter paper, Sri Lanka, children

INTRODUCTION

Iron deficiency anemia continues to be a public health challenge, particularly in developing countries (1–3). Serum ferritin is a sensitive index of depleted body iron stores (4–6), with concentrations <12–15 μg/L usually reflecting iron deficiency (5, 6). Novel approaches were reported for assessing iron status in the field with use of spot methods to analyze ferritin (7–9). We showed that serum samples obtained from venous blood that were spotted and air-dried on filter paper could be kept at room temperature for ≤4 wk before analysis and yield concentrations that are not significantly different from those obtained by the traditional method (7). A plasma spot method was reported that provided results that were comparable to those of traditional plasma ferritin analyses, with spot samples that could be stored at room temperature for ≤2 wk or stored refrigerated for ≤4 wk before analysis (8, 9). The potential advantages of spot methods for analyzing ferritin from serum and plasma samples that are spotted and dried on filter paper (7–9) are ease in collection, handling, storage, and transportation and greater safety against blood-borne pathogens. In addition, these spot methods do not require a cold chain (ie, refrigeration or freezing) during the collection and storage of samples for ≤4 wk (7) before ferritin analysis. Because spot methods do not require a cold chain, the feasibility of assessing serum ferritin in remote areas is increased.

The spot methods have not been evaluated in field settings in developing countries. Furthermore, spot methods have only been analyzed with serum or plasma obtained from venous blood. Venous blood collection, however, is impractical in field situations because it requires trained phlebotomists to draw blood. Venipuncture may also be limited further in field settings because of cultural and age constraints. Some of these concerns can be overcome by collecting capillary blood samples by finger prick. However, to our knowledge, the use of capillary blood for assessing iron status by using spot methods has not been examined. Thus, in the present study we sought to *1*) evaluate the validity of a dried-serum-spot (DSS) ferritin method by using sera from venous and capillary blood collected from school-aged

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children in Colombo, Sri Lanka (7) and 2) compare the DSS method with the traditional method for ferritin analysis on venous serum that was stored frozen until analysis.

SUBJECTS AND METHODS

Before addressing the main objective of the study, we first evaluated whether DSS samples prepared in the humid climate of Colombo, Sri Lanka, would require storage with desiccant to yield accurate ferritin results (study 1).

Study 1: effect of storing venous dried-serum-spot ferritin samples with or without desiccant

Subjects

Twenty-one healthy volunteers (15 females and 6 males) aged 20–25 y provided informed consent to participate in the present study, which followed the protocols approved by the Ethical Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka.

Blood collection and analyses

Venous blood (1 mL) was drawn by a trained nurse with the use of a sterile syringe and needle. The blood samples were centrifuged (481 × *g*, 8 min, 28°C) to obtain serum. DSS samples were prepared by first pipetting 4–6 venous serum spots (20 μL each) onto Whatman no. 1 filter paper (Whatman Inc, Clifton, NJ). Samples were then air-dried and 2–4 venous DSS samples per subject were placed in an airtight polyethylene bag (Ziploc; DowBrands, Indianapolis). The remaining 2 venous DSS samples per subject were placed in a second air-tight polyethylene bag (Ziploc; DowBrands). The second set of bags with samples from 7–8 subjects (each bag containing 2 venous DSS samples per subject) was then placed into another larger airtight polyethylene bag containing calcium sulfate as a desiccant (WA Hammond Drierite Co, Xenia, OH). The DSS samples for 7–8 subjects, stored with or without desiccant, were then shipped to Penn State University, University Park, PA, without a cold chain for ferritin analysis by the spot ferritin method. An aliquot (100 μL) of venous serum was frozen for each subject. A single package of frozen serum samples for all subjects was shipped on dry ice to Penn State University for ferritin analysis by the traditional radioimmunoassay method (Diagnostic Products Corporation, Los Angeles).

Spot ferritin method

The spot ferritin method according to Ahluwalia et al (7) was run with some modifications to analyze ferritin in DSS samples that were stored at room temperature for 2 wk from the date of collection. In the present study, cellulase from *Trichoderma reesei* (Sigma Chemical Co, St Louis) was used and the DSS samples were digested with cellulase for 6 h. On the day of the assay, spot samples kept at room temperature were cut around the spot circumference, placed in glass vials with 750 μL 1% ammonium acetate buffer (pH = 5.0) containing 235 U cellulase (obtained from *T. reesei*; Sigma Chemical Co), incubated in a water bath at 37°C for 6 h, transferred to microcentrifuge tubes (pH = 8), and refrigerated until the next day. The samples were then centrifuged at 481 × *g* for 8 min at 28°C and ferritin was assayed by radioimmunoassay (Diagnostics Products Corporation) in duplicate in 200 μL of supernatant fluid. In each spot ferritin run, an internal control of a DSS sample prepared with pooled serum was also included for quality-control purposes. The ferritin concentration for this internal control by the traditional method over 6 runs was 118 ± 6.6 μg/L and the batch-to-batch CV was 5.6%. With the DSS ferritin method, over 7 runs, the ferritin concentration for the internal control was 115 ± 7 μg/L and the batch-to-batch CV was 6.1%.

Study 2: validity of venous and capillary dried serum spot ferritin

Subjects

One hundred healthy schoolchildren (56 females and 44 males) aged 8–10 y were recruited for participation in the present study after permission was obtained from the Ministry of Higher Education and from the principals of schools. Written, informed consent was obtained from the parents or guardians of the children after the study protocols were approved by the Ethical Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka. On the basis of clinical examination and self-reported medical history, children with infections or inflammation ≤3 wk before recruitment, hepatosplenomegaly, systemic diseases (eg, renal or liver disease), or osteomyelitis were excluded.

Blood collection and analyses

Venous blood (1 mL) was drawn by a trained nurse with the use of a sterile syringe and needle. A portion of the venous blood was placed into a tube containing EDTA for estimation of hemoglobin by the cyanmethemoglobin method (Procedure no. 525-A; Sigma Diagnostics St. Louis). A trained laboratory technician obtained capillary blood samples (200–300 μL) by finger prick and collected the samples into three 100-μL microcapillary tubes. Blood samples were brought to the laboratory and centrifuged (481 × *g*, 8 min, 28°C) to obtain serum. Precisely 20 μL per spot venous and capillary serum were pipetted onto Whatman no. 1 filter paper (Whatman Inc) and air-dried. DSS samples for each subject were placed in airtight plastic bags (Ziploc; DowBrands) and shipped to Penn State University for ferritin analysis by the spot ferritin method (described above). DSS samples were not stored with desiccant for this study because the results of study 1 showed that storage of DSS samples with or without desiccant yielded ferritin results that were not significantly different from those obtained by the traditional ferritin method (details provided in Results). The remaining venous serum was stored at −20°C and sent to Penn State University on dry ice for analysis of serum ferritin by the traditional method of radioimmunoassay (Diagnostic Products Corporation).

Statistical analyses

All analyses were conducted by using the Statistical Analysis System (SAS Institute, Cary, NC) on an IBM personal computer (Dell Computer Corporation, Austin, TX) (10). Data were logarithmically transformed because serum ferritin concentrations are consistent with a log-normal distribution.

In study 1, the effect of storing DSS samples with or without the desiccant calcium sulfate was evaluated by using paired *t* tests with Bonferroni's correction to determine the significance of the differences between ferritin values obtained by the spot ferritin method for DSS samples stored with or without desiccant and values obtained by the traditional method. Pearson's product-moment correlation coefficient was computed to examine the relation between the traditional method and the spot ferritin method on DSS samples stored with or without desiccant. The

TABLE 1
Serum ferritin concentrations measured by the traditional method and the dried-serum-spot (DSS) method with venous DSS samples stored with or without desiccant

Method	Ferritin	Correlation with traditional method *(r)*
	μg/L	
Traditional (n = 21)	24.2 (10.4–56.4)[1]	—
Venous DSS		
With desiccant (n = 21)	28.7 (13.5–61.0)	0.88[2]
Without desiccant (n = 21)	25.0 (10.2–61.1)	0.90[2]

[1] Geometric mean; ±1 SD in parentheses. Means were not significantly different from each other.
[2] P = 0.0001.

relation between the traditional method and the spot ferritin method was evaluated with linear regression analysis. The line of regression was tested against the line of unity (slope = 1, intercept = 0) by simultaneously testing the hypotheses of slope = 1 and intercept = 0 (7). Analysis of variance was conducted to examine the differences between ferritin values obtained by the traditional method and the spot ferritin method, with storage condition and sample identification used as the main effects. Post hoc analysis was conducted by using Tukey's test.

In study 2, the validity of venous and capillary DSS ferritin assays was evaluated by using paired t tests with Bonferroni's correction to test the significance of differences between ferritin values measured by the spot ferritin method (that used venous and capillary DSS samples) and values measured by the traditional method. Pearson's product-moment correlation coefficient was computed to examine the relation between the traditional method and the spot ferritin method for both venous and capillary DSS samples. The relation between the values obtained by the traditional method and the spot ferritin method for venous and capillary DSS samples was evaluated with linear regression analysis, as described for study 1. Analysis of variance was conducted with source of serum (venous or capillary) and sample identification as the main effects. Post hoc analysis was conducted by using Tukey's test. We used the approach of Bland and Altman (11) to compare the spot ferritin method for venous DSS samples with the traditional method by computing the difference in ferritin values against the mean ferritin value obtained by both methods for each subject (7). The same approach was repeated for capillary DSS samples.

RESULTS

Study 1: effect of storing venous dried-serum-spot samples with or without desiccant

The mean ferritin values obtained by the traditional method and by the spot ferritin method for venous DSS samples stored with or without desiccant were not significantly different (**Table 1**). Venous DSS ferritin, irrespective of storage conditions was strongly correlated with the traditional ferritin method (Table 1, P = 0.0001). Furthermore, the slope and intercept of the line of regression for the spot ferritin method (irrespective of DSS storage condition) were not significantly different from the line of unity (slope = 1 and intercept = 0). When subjects were classified as either iron deficient or iron sufficient on the basis of ferritin values measured by the traditional method, there were 6 iron-deficient (serum ferritin <15 μg/L) and 15 iron-sufficient (serum ferritin ≥15 μg/L) participants in the study cohort. For both storage conditions, the DSS ferritin method accurately classified individuals as iron-deficient or iron-sufficient. Specifically, 4 of 6 (67%) iron-deficient and 14 of 15 (93%) iron-sufficient subjects were correctly classified by use of the venous DSS ferritin method that used samples stored with desiccant. The percentage of subjects correctly classified by the venous DSS ferritin method that used samples stored without desiccant was higher: 83% for iron-deficient and 100% for iron-sufficient subjects.

Study 2: validity of venous and capillary dried-serum-spot ferritin method

The mean (± SD) hemoglobin concentration of the study cohort was 117.8 ± 8.4 g/L. Thirty-two percent (n = 32, 16 females and 16 males) of the subjects were anemic (hemoglobin <115 g/L) and 6% had serum ferritin <15 μg/L.

The mean ferritin values by the 3 methods (traditional and venous and capillary DSS ferritin methods) did not differ significantly (**Table 2**). Both venous and capillary DSS ferritin methods correlated strongly with the traditional method (Table 2 and **Figure 1**). Furthermore, when tested against the line of unity (slope = 1 and intercept = 0), the slope and intercept of the regression lines for venous and capillary DSS ferritin methods were not significantly different from 1 and 0, respectively (Figure 1).

We examined the relation between the spot ferritin method for venous and capillary DSS samples with the traditional method in the range of 0–50 μg/L because the utility of measuring serum ferritin for assessing iron depletion lies on the lower end of the distribution. The spot ferritin and traditional methods remained highly correlated, even in this lower range: r = 0.85 and P = 0.0001 for the venous DSS and traditional methods and r = 0.81 and P = 0.0001 for the capillary DSS and traditional methods.

The mean difference in ferritin values (ferritin values obtained by the venous DSS ferritin method − ferritin values obtained by the traditional method) was −5.8 μg/L. Thus, the venous DSS method on average yielded ferritin values that were 5.8 μg/L lower than values obtained with the traditional ferritin method. When this analysis was restricted to subjects with ferritin values <50 μg/L, as determined by the traditional method, the mean difference in ferritin values obtained by the 2 methods was only −3.42 μg/L (**Figure 2**).

TABLE 2
Serum ferritin concentrations measured by the traditional method and the dried-serum-spot method with venous or capillary blood

Method	Ferritin	Correlation with traditional method *(r)*
	μg/L	
Traditional (n = 100)	33.1 (18.6–58.8)[1]	—
DSS		
Venous (n = 100)	26.9 (15.3–47.4)	0.88[2]
Capillary (n = 99)	33.9 (20.9–54.8)	0.86[2]

[1] Geometric mean; ±1 SD in parentheses. Means were not significantly different from each other.
[2] P = 0.0001.

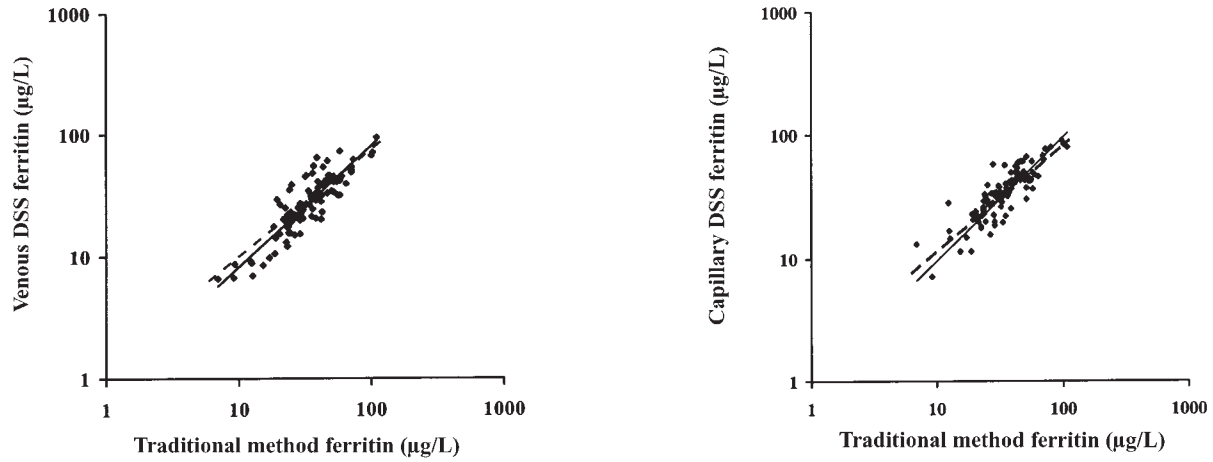

FIGURE 1. Correlations between serum ferritin values obtained by the traditional method and by the dried-serum-spot method (DSS) that used venous or capillary blood. In both panels, the regression line (dashed line) is compared with the line of unity (solid line) with a slope of 1 and intercept of 0. The regression equation was log y = 0.99(log x) − 0.07 (R^2 = 0.77) for venous DSS and log y = 0.84(log x) + 0.25 (R^2 = 0.74) for capillary DSS samples.

The mean difference in ferritin values (ferritin values obtained by the capillary DSS ferritin method − ferritin values obtained by the traditional method that used serum samples stored frozen until analysis) was not significant (0.03 μg/L). When this analysis was restricted to subjects with ferritin values <50 μg/L by use of the traditional method, the mean difference in ferritin values obtained by the 2 methods was 2.41 μg/L (Figure 2).

By using serum ferritin values <15 μg/L to define iron deficiency (5, 6), we determined that there were 6 iron-deficient and 94 iron-sufficient children in this study cohort. By using the

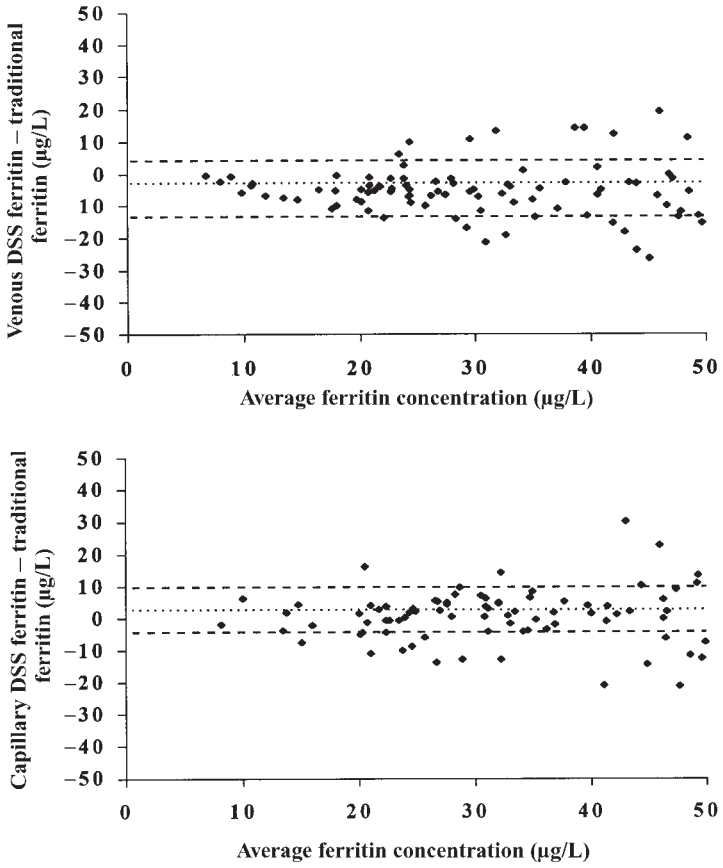

FIGURE 2. Relation between the differences in serum ferritin values obtained by the dried-serum-spot (DSS) ferritin method (for venous or capillary blood) and the traditional method against the average of values obtained by the 2 methods for samples with serum ferritin values between 0 and 50 μg/L. In each panel, the dotted and dashed lines represent the mean (± SD) of the difference in ferritin values obtained by the 2 methods.

venous DSS ferritin method, all 6 iron-deficient subjects were correctly identified as iron-deficient, and 88 of 94 iron-sufficient subjects had DSS ferritin values ≥15 μg/L. The corresponding numbers for the capillary DSS ferritin method were slightly lower: 80% iron-deficient and 97% iron-sufficient subjects were correctly identified with the use of the capillary DSS method.

DISCUSSION

One of the challenges in addressing global micronutrient deficiencies is the availability of sensitive and specific assessment tools that are applicable for field use in remote areas. Novel approaches, such as analyzing ferritin from serum or plasma samples that are spotted and dried on filter paper, were reported to yield accurate and reliable results (7–9). Recently, assessment of vitamin A status on the basis of serum retinol measurement from capillary-blood spot samples was also reported (12). Because the spot ferritin methods had not been previously evaluated in field settings and were established by using serum or plasma obtained from venous blood, we conducted the present study to evaluate the accuracy of a spot ferritin method (7) that uses DSS samples obtained from venous and capillary blood in school-aged children living in Colombo, Sri Lanka.

Because Colombo, Sri Lanka, has a humid climate, we first examined whether DSS samples obtained in such an environment would need to be stored with desiccant (calcium sulfate) to yield accurate ferritin results. We found that DSS sample storage with or without desiccant yielded ferritin values that did not differ significantly from the values obtained by the traditional ferritin method. Furthermore, there was a high correspondence between DSS ferritin, irrespective of storage condition, and the traditional method (r ranged from 0.88 to 0.90). Thus, DSS samples were not stored with desiccant in the primary study because storage with desiccant involves an additional step and extra shipping costs.

The main purpose of the present study was to evaluate the validity of a spot method for analyzing ferritin (as an index of iron status) from venous and capillary serum samples dried on filter paper compared with the traditional method (as the reference method or gold standard) in a field setting. Several approaches were taken to examine and establish the concordance between the spot and traditional ferritin methods. First, both venous and capillary DSS ferritin methods yielded ferritin values that did not differ significantly from serum ferritin values obtained by the traditional method. Second, consistent with our previous report (7), the venous spot ferritin method correlated highly with the traditional method (r = 0.88); capillary DSS ferritin, examined for the first time in a field setting, was also strongly correlated (r = 0.86) with the traditional ferritin method. Third, the venous and capillary DSS methods had a high degree of agreement with the traditional ferritin method because the lines of regression with the traditional method were not significantly different from the line of identity ($y = x$). This was further substantiated by using the approach of Bland and Altman (11). The differences between the DSS ferritin methods and the traditional ferritin method for venous and capillary spots were small (5.8 μg/L for venous DSS and 0.1 μg/L for capillary DSS) for the entire study cohort.

It could be argued that the agreement of the venous and capillary DSS methods with the traditional method appeared stronger in samples on the lower end of the serum ferritin distribution than in samples with higher ferritin concentrations. To address this further, we examined the correlation of the DSS methods with the traditional method in samples with serum ferritin <50 μg/L and ≥50 μg/L separately. First, the strength of these relations was not affected by ferritin concentrations and the correlations remained high (≥0.8) for samples with traditional ferritin concentrations <50 or ≥50 μg/L. Second, there was no significant relation between the difference in ferritin concentration values obtained by the capillary and venous DSS methods and those obtained by the traditional method (eg, venous DSS method − traditional method) for samples with serum ferritin <50 or ≥50 μg/L. Thus, the apparent trend of greater agreement on the lower end of the serum ferritin concentration distribution was not significant. This relation should be examined further in future studies because there were only 20 samples with serum ferritin ≥50 μg/L in the present study.

It is interesting to note that although the DSS and traditional methods had a high degree of concordance, some outliers exhibited much variation in terms of difference in ferritin values between the DSS and traditional methods (Figure 2). This spread could not be fully explained by the analytic error in the spot ferritin method because the within-assay CV for replicates ranged from 3% to 5% for venous and capillary DSS ferritin. Although efforts were made to use a well-calibrated pipet, variation in the volume of serum dispensed for DSS preparation could have also contributed to the measurement error in these cases. The variance for capillary DSS samples also could not be completely explained by hemodilution because the scatter of differences in ferritin values between the DSS and traditional methods was similar for venous and capillary DSS. In future studies, outliers may be further reduced by greater precision in collecting blood, preparing the spots, and carrying out the spot ferritin assay.

The present study was designed to evaluate the accuracy of a spot method for classifying children as iron deficient or sufficient. The prevalence of iron deficiency (serum ferritin <15 μg/L) in this healthy cohort of school-aged children, however, was smaller (6%) than what was projected. The partition of subjects designated as iron sufficient and deficient was similar with the use of the DSS ferritin and traditional methods. We could not evaluate the sensitivity and specificity for the venous and capillary DSS ferritin methods with sufficient confidence because of the small number of iron-deficient subjects in the present study.

In conclusion, the analysis of ferritin with the use of the spot ferritin method in a field setting accurately reproduced the results reported in a laboratory setting (7), and showed that both capillary and venous DSS methods are valid for ferritin analysis. In addition, the capillary DSS method is a less invasive and practical approach for ferritin measurement. Further studies in populations with a high prevalence of iron deficiency are needed to confirm these promising findings. Studies are currently underway to establish and validate simpler techniques for preparing DSS samples from capillary blood in field settings.

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