Ferritin concentrations in dried serum spots prepared by standard compared with simplified approaches: a validation study in Guatemala City¹–³

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ABSTRACT

Background: Spot ferritin assay on dried serum spot (DSS) samples provides reliable and accurate assessment. Standard DSS preparations, however, involve precise serum aliquots and require some skill and training of field personnel.

Objective: We evaluated the validity of the spot ferritin assay on DSS samples prepared by simplified approaches and standard technique in Guatemala City.

Design: Venous blood (5 mL) was obtained from 104 subjects aged 24 ± 15 y (x ± SD) and transferred into nonheparin-containing (2 plain and 2 self-sealing) capillary blood collection tubes. Three DSS samples were prepared: A (standard, 20 μL serum), B (blot, ≈30–35 mm serum column), and C (dispenser, 20 μL serum pushed directly from self-sealing capillary tubes with a dispenser). Spots were air-dried and placed in hermetic plastic bags with a desiccant. Two weeks later, entire spots for DSS A and C samples and a circle in the center for DSS B samples were analyzed.

Results: DSS ferritin A, B, and C correlated strongly with traditional ferritin (r = 0.71–0.88, P < 0.001). The geometric mean (± 1 SD and +1 SD) values for the DSS A, B, and C and traditional ferritin methods were 27.5 (12.6, 60.2), 32.4 (13.5, 77.6), 27.5 (11.7, 64.6), and 30.2 (13.8, 66.1) μg/L, respectively, and did not differ significantly. The difference in ferritin values by various DSS approaches compared with the traditional approach was small (<4 μg/L; P > 0.05).

Conclusions: Simplified and standard DSS methods provide accurate iron-status assessment in population studies. The simplified DSS approaches for serum ferritin measurement need to be evaluated further in populations in whom iron deficiency is prevalent. Am J Clin Nutr 2005;81:1366–71.

KEY WORDS Iron-status assessment, ferritin, dried serum spots, filter paper approach, field studies

INTRODUCTION

Micronutrient deficiencies continue to be a public health problem worldwide (1, 2). The recently developed technique of applying dried spots of serum, plasma, or blood to filter paper for the assessment of iron, vitamin A, and folic acid status (3–8) may assist in the evaluation of micronutrient status in population studies.

We and others have shown that dried serum spot (DSS) or plasma spot ferritin assays can provide an accurate and reliable measurement of ferritin (3–5, 9). In addition, the advantage of these methods is that the samples are easy to collect, handle, store, and transport and they provide greater safety against certain blood-borne pathogens (3–5, 9, 10). Moreover, our DSS ferritin method does not require a “cold chain” (refrigeration or freezing) for samples collected and stored for up to 4 wk (3) before ferritin measurement, which makes this method attractive for use in remote settings. Furthermore, in a field setting with young children in Colombo, Sri Lanka, we showed that capillary blood can be used to prepare DSS samples to yield accurate and reliable measurements of ferritin (9).

The DSS ferritin method has been validated for applying a precise volume of serum onto filter paper. This requires prior separation of serum, followed by the preparation of DSS by personnel with skill and training in accurate dispensing with the use of a precision pipette. We were interested in developing simplified approaches for preparing DSS samples for iron-status assessment in population studies that would require minimal skill and training of fieldworkers. In pilot studies, we identified 2 approaches for preparing DSS samples that could be suitable for field use: 1) blotting a large drop of serum (≈30–35 mm) and 2) using a dispensing pipette (SAFEPETTE; SAFE-TEC Clinical Products Inc, Iviland, PA) that delivers a fixed (20 μL) volume of serum from self-sealing capillary tubes (SAFECAP; SAFE-TEC Clinical Products Inc) without having to file and break off the tube to separate the serum. Thus, the objective of this study was to evaluate the validity of the DSS ferritin method with the use of these simplified, more user-friendly DSS techniques against the traditional method for ferritin measurement on venous serum stored frozen. The study was conducted in a periurban setting in Guatemala City. Because the filter paper spot methods have not been evaluated extensively in field settings in developing countries, a secondary objective of this study was to evaluate the DSS ferritin method (using standard DSS made by a precise aliquot of 20 μL serum) under humid conditions in Guatemala City.

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SUBJECTS AND METHODS

Subjects

One hundred four apparently healthy subjects aged 24 ± 15 y (x ± SD) were recruited in a periurban community (Ciudad Peronia) located ≈7 km from Guatemala City. Written informed consent was obtained after the protocols were approved by the Human Subjects Committee of CeSSIAM in Guatemala and the Office of Research Compliance at the Pennsylvania State University. On the basis of clinical examination and self-reported medical history, subjects with infections or inflammation in the 3 wk before recruitment were excluded.

Blood collection and analyses

Venous blood (5 mL) was drawn with a disposable sterile syringe and needle. A portion of venous blood was used to fill 6 different capillary tubes: 2 heparin-containing capillary tubes (VWR Scientific Inc, St Paul, MN), 2 “plain” capillary tubes with no anticoagulant (internal diameter = 1 mm; VWR Scientific Inc), and 2 self-sealing capillary tubes with no anticoagulant (SAFCAP, SAFE-TEC Clinical Products Inc). The remaining blood was transferred to an evacuated tube with no anticoagulant (Figure 1). At the community center, capillary tubes were centrifuged at 13 460 × g (11 500 rpm) for 5 min at 25 °C in a microcentrifuge (International Equipment Centrifuge, Woburn, MA), and the hematocrit was read with the use of a microcapillary reader (International Equipment Centrifuge). Those subjects who were found to be anemic were provided iron supplements. Centrifuged capillary tubes were brought back to the CeSSIAM laboratory for the preparation of DSS samples as described below. Blood samples in the evacuated tubes that were brought back to the CeSSIAM laboratory were also centrifuged with a conventional centrifuge (4650 × g, or 8650 rpm, for 5 min) to obtain serum. Serum was stored at −20 °C and shipped by 3-d courier to Pennsylvania State University on dry ice for analysis of serum ferritin with a traditional radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA).

Preparation of dried serum spot samples

Serum spots were prepared on filter paper (Whatman #1; Whatman Inc, Clifton, NJ) and air-dried overnight at room temperature. For each subject, 2 DSS samples were prepared for each approach described below. For DSS A (standard spot), precisely 20 μL serum per spot was pipetted with a calibrated precision pipette. For DSS B (blotted spot), after centrifugation, the plain capillary tubes were scored above the buffy coat with a file and broken off to separate serum column in capillary tube. Serum was blotted directly on filter paper from the column to obtain a circle of 30–35 mm in diameter (DSS B). For DSS C (using dispenser), the centrifuged self-sealing capillary tube containing the blood clot and serum was inserted into its accompanying dispenser (SAFEPETTE; SAFE-TEC Clinical Products Inc), which was precalibrated by the manufacturer to deliver a fixed volume of serum (20 μL). This dispenser uses the red blood cell clot to directly push the serum out of the capillary tube, which overcomes the need to separate the serum column from blood cells by scoring and breaking off the capillary tube.

DSS samples A, B, and C were placed in hermetic plastic zip closure bags (Ziploc; DowBrands, Indianapolis, IN); weighing paper (Fisher Scientific, Philadelphia, PA) was used to separate individual DSS samples. The set of polyethylene bags was placed into another larger hermetic polyethylene zip-closure bag containing ≈20–25 g CaSO₄ as a desiccant (WA Hammond Drierite Co, Xenia, OH) as a prudent measure to restrict humidity. The DSS samples from 15–20 subjects were sent to Pennsylvania State University, University Park, PA, without a cold chain and
within 1 wk of preparation by 3-d courier for analysis by the DSS ferritin method (9). The DSS samples were kept at room temperature and were analyzed 2 wk from the date of collection, at Pennsylvania State University, by using the spot ferritin assay of Ahluwalia et al (9). For the DSS B samples, a circle (diameter = 15.2 mm, corresponding to 20 μL serum; established from a preliminary study of 56 subjects in which 3–4 standard spots were prepared per subject and 3 diameter measurements were taken per spot) in the center of the spot and for DSS A and DSS C the entire spots were analyzed. Each spot ferritin run included DSS samples prepared by the 3 different approaches for each subject as well as 2 internal controls of DSS samples prepared by using pooled serum samples with ferritin concentrations of 30 and 100 μg/L, respectively. The within-assay and batch-to-batch assay CVs for the control pooled serum samples were 5.8% and 7.5%, respectively. The analytic CVs for replicates, for the DSS ferritin method using DSS A, B, and C samples, were similar (<6%).

Statistical analyses

The analyses were carried out by using SAS version 8 (SAS Institute, Cary, NC) on a personal computer (Dell Computer Corporation, Austin, TX) (11). Data were logarithmically transformed because serum ferritin is consistent with a log-normal distribution.

The validity of the DSS ferritin method on samples prepared by using various approaches (standard, blot, and dispenser) were evaluated by using paired t tests with Bonferroni’s correction to test the significance of differences between ferritin values determined with the DSS ferritin method and values obtained by the traditional method. Pearson’s product-moment correlation coefficients were computed to examine the relation between the traditional method and the DSS ferritin method for DSS samples prepared with the 3 techniques described. Fisher’s z transformation of the r coefficients, with Bonferroni’s correction, was used to test the significance of differences between the coefficients. The relation between the traditional method and the DSS ferritin method on DSS samples prepared with the 3 approaches was also evaluated with linear regression analysis. An analysis of variance was carried out with the DSS sample preparation technique (standard, blot, and dispenser) and sample identification number as main effects. We followed the approach of Bland and Altman (12) to compare the DSS ferritin method on DSS samples (prepared with the standard approach) with the traditional method by computing the difference in ferritin values against the mean ferritin value determined with both methods for each subject (3). The same approach was repeated for DSS B and for DSS C samples.

RESULTS

The study cohort consisted of 87 women and 17 men aged 24 ± 15 y (x ± SD), who had a hematocrit of 0.41 ± 0.03 (x ± SD). Data for 4 subjects (2 men and 2 women), obtained with the traditional ferritin method, were not available because their serum samples hemolyzed; the final data set consisted of 100 subjects. Eight percent of the subjects were anemic, whereas 16.0% had a serum ferritin concentration <15 μg/L.

The mean ferritin values obtained with the 4 methods (traditional method and DSS A, B, and C methods) did not differ significantly (Table 1). All 3 approaches of the DSS ferritin method showed a highly significant correlation with the traditional method (Table 1 and Figure 2; P = 0.0001). The strongest correlation between the traditional and the DSS ferritin method was noted for the DSS A approach (r = 0.88). The correlation between the DSS B approach and the traditional method (r = 0.71) was significantly lower (z = 3.45, P < 0.001) than the correlation between the DSS A approach and the traditional method (r = 0.88). However, the correlation between the DSS C approach and the traditional method (r = 0.84) did not differ significantly (z = 1.1, P > 0.05) from the correlation between the DSS A approach and the traditional method (r = 0.88).

The mean differences in ferritin values by the DSS ferritin method and the traditional method were small: −3.6, −2.7, and −1.6 μg/L for DSS A (standard spot), DSS B (blot), and DSS C (dispenser), respectively (P > 0.05). Because the utility of measuring serum ferritin to assess iron depletion lies on the lower end of the distribution, we examined this difference in the range of 0–50 μg/L. When the analysis was restricted to subjects with ferritin values < 50 μg/L, determined with the traditional method, the mean difference in ferritin values determined with the DSS ferritin method compared with the traditional method, remained small and nonsignificant (P > 0.05): −6.0 μg/L (with the DSS A approach; data not shown), 7 μg/L (with the DSS B approach; Figure 3), and 1.38 μg/L (with the DSS C approach; Figure 3). Thus, the DSS ferritin method using simplified approaches yielded ferritin values that, on average, did not differ significantly from ferritin values determined with the traditional method.

On the basis of a serum ferritin concentration <15 μg/L indicating iron deficiency (13, 14), there were 16 iron-deficient and 84 iron-sufficient subjects in the study cohort. The DSS A approach classified subjects accurately as iron-deficient and iron-sufficient (93.8% sensitivity and 96.3% specificity). The sensitivities of the DSS B and DSS C approaches were relatively lower, 71% and 75%, respectively; the specificity of these methods remained high (98% for DSS B and 90% for DSS C).

DISCUSSION

One of the challenges in addressing global micronutrient deficiencies is the availability of sensitive and specific assessment tools for use in field situations. Most large-scale studies and national surveys use measurements of hemoglobin or hematocrit, singly or in combination, to establish anemia. This approach...
provides important indications regarding the prevalence of anemia; however, it does not provide any information on the contributing causes. Moreover, both hemoglobin and hematocrit measurements are neither sensitive nor specific indicators of iron status (15). Iron deficiency is a leading cause of anemia in developing countries, particularly during periods of enhanced iron needs related to growth and during the reproductive years for women. Therefore, establishing the cause of anemia is important for determining suitable intervention approaches, including improvements in iron intake if indicated.

An important advance in this respect is the development and validation of various filter paper–based spot assays that have been shown to provide results that are as reliable and precise as those of traditional methods for measuring serum ferritin (3–5, 8, 9). Indeed, the use of our DSS ferritin method (3) in an isolated group of indigenous young women in northern Mexico by Monárrez-Espino et al (16) showed that anemia in this cohort was primarily attributable to iron deficiency.

The spot assays offer several advantages related to the handling, transport, and storage of DSS samples. The spot assays do not require a cold chain, and the risk of transmission of certain blood-borne pathogens is virtually eliminated (3, 4, 9, 10) with this method. The filter paper–based assays for ferritin analysis, however, require the delivery of precise volumes of serum onto filter paper via precision pipetting devices, which in turn necessitates a higher degree of technical skill and training for field staff (4). Because we and others have shown that whole-blood spots are not valid indicators for measuring serum ferritin (3, 4), one has to rely on serum for ferritin measurements. Therefore, interest in simplifying approaches for the preparation of DSS samples has developed, and preliminary findings on the preparation of DSS samples with the use of a syringe to expel serum out of
centrifuged capillary tubes and recording the length of the column dropped have been reported (17). This approach, however, still requires considerable precision and some training for the field staff. Thus, our interest was to identify other ways to simplify the process of preparing DSS samples that could render the DSS ferritin assays more user-friendly.

In pilot experiments we established that serum could be blotted onto filter paper from the capillary tube, yielding a circle of 30–35 mm in diameter; then, at the time of analysis, a circle of a diameter equaling 15.2 mm (the average diameter of the standard DSS prepared by precisely pipetting 20 μL serum; n = 56; 3–4 spots per subject) could be cut (or punched) and analyzed by using the DSS ferritin method described previously (9). We also identified a dispenser system (see Subjects and Methods) that uses precisely fitting self-sealing capillary tubes to dispense serum from the centrifuged capillary sample by using the red blood cell clot itself to push out a precise volume of serum onto filter paper. We explored this dispenser and were able to consistently produce serum spots of 20 μL (n = 30; CV < 3%; N Aahuwalia and J Bulux, unpublished observations, 1999). The interest in using the dispenser system was to overcome the need to score and break off the capillary tube above the red blood cell anduffy coat level in field situations and dispensing serum directly from the centrifuged capillary tube. This approach makes the preparation of DSS samples simpler and may also improve safety by overcoming the small risks associated with the process of breaking the capillary tube containing cells and serum. Because the recently developed DSS ferritin methods have been evaluated in only a few settings (9), a secondary objective was to evaluate the validity of standard DSS approaches (prepared with precise pipetting of 20 μL serum) in this community setting.

The main purpose of the study was to evaluate the DSS ferritin method by using DSS samples prepared by standard (DSS A) and simplified [ie, by blotting serum (DSS B) and by using a dispenser system (DSS C)] approaches against the traditional ferritin method using serum samples stored frozen until analyzed. DSS A, B, and C samples were stored with desiccant, with no cold chain, in hermetic plastic zip closure bags and analyzed 2 wk later with a DSS ferritin assay (9). The results from this study, which was conducted in a humid setting, confirm our previous findings in Colombo, Sri Lanka (9), that the standard DSS prepared by precise pipetting of 20 μL serum (ie, DSS A) provided an accurate assessment of iron status compared with the traditional ferritin method, which was used as the standard of reference. The mean ferritin concentrations determined with the 2 methods did not differ significantly, the methods were highly correlated (R² = 0.77), and the classification of subjects as iron-deficient or iron-sufficient was accurate (93.8% sensitivity and 96.3% specificity).

Of greater interest in the current study, however, was the comparison of the 2 newer simplified approaches of DSS preparation for use with the DSS ferritin assay against the gold standard of traditional ferritin determination. The DSS ferritin method on spots prepared by using the simplified techniques described above [ie, blotting a large circle of serum (DSS B) and delivering a fixed volume (20 μL) of serum directly from the centrifuged self-sealing capillary tube with the use of a dispenser system (DSS C)] provided an accurate measurement of the analyte. Mean ferritin values measured by using these simplified methods did not differ significantly from those obtained with the traditional ferritin method (P > 0.05). The DSS ferritin method that used the simplified approaches correlated significantly with the traditional method, although the strength of the correlation was smaller with the DSS B approach than with the DSS A approach. Finally, we found no bias (with the Bland and Altman approach) in the spread of the differences in ferritin values determined with the DSS ferritin method that used simplified approaches and the traditional ferritin method (Figure 2). Although there were few outliers, the mean differences in ferritin values determined with the DSS B and C approaches compared with the traditional ferritin method were small (−2.7 and −1.6 μg/L with the DSS B and DSS C approaches, respectively). Therefore, our findings suggest that the DSS ferritin method using DSS samples prepared by using these simplified approaches (ie, DSS B and DSS C) offers a feasible and accurate alternative to the standard DSS approach (ie, DSS A, which requires a precise aliquot of serum) for the assessment of iron status in a population-based survey whenever a simplified field approach is needed.

In future surveys, we recommend preparing DSS samples with the use of self-sealing capillary tubes with a dispenser system, because this method provides not only an accurate determination of serum ferritin but also is simpler that blood samples—once centrifuged in self-sealing capillary tubes—can be simply inserted into the dispenser to push out serum directly onto filter paper. This approach overcomes the somewhat cumbersome step of scoring traditional glass capillary tube above the buffy coat level to obtain the serum column first, followed by delivery of serum onto filter paper. The removal of this step makes the dispenser system not only simpler but also safer for preparing DSS samples for ferritin determination. In situations in which the dispenser system cannot be used because of financial or other constraints, an alternative accurate DSS method would be the blot method. With the blot method, blood is collected into traditional capillary tubes and centrifuged. The serum is then separated, and DSS samples are prepared by blotting a large circle onto filter paper for the analysis of ferritin. Thus, the choice for use of these simplified methods would depend on the situation and the resources available. We previously showed that capillary blood can be collected in field settings to prepare DSS samples and yield accurate ferritin measurements (9). We believe that these findings in finger-stick capillary blood from young children in Sri Lanka can be extended to the simplified preparation of DSS samples described in this study. Future studies are needed in field situations to validate the simplified methods for DSS sample preparation for the measurement of ferritin in finger-stick blood samples.

In terms of assessing iron status at an individual level, the standard DSS ferritin method (ie, DSS A) appeared more sensitive than the DSS ferritin methods that used simplified approaches (ie, DSS B and DSS C). Because this study was not designed to evaluate the sensitivity and specificity of the DSS methods, these findings are preliminary. It is recognized that the sensitivity estimates are influenced by the prevalence of the condition as well as the choice of the cutoffs used (18). In this small study, the prevalence of anemia was only 8% and that of iron deficiency was 16%. We also modeled the sensitivity estimates for the various DSS approaches by using a higher cutoff for serum ferritin, ie, 50 μg/L. In fact, this cutoff may have been more suitable in the current study cohort, in whom subclinical inflammation may have been present (15, 19). The sensitivity of both simplified approaches was indeed improved when this higher cutoff was used (85.9% and 95.4% with the DSS B and
DSS C approaches, respectively). Thus, further studies are needed to evaluate the utility of these simplified DSS approaches in groups in whom iron deficiency is more widespread.

Practical and scientific issues, such as the choice of filter paper and the use of desiccant, deserve consideration in future studies and surveys of the DSS ferritin method. We used Whatman #1 filter paper in the current study because our initial method was established and validated with this type of filter paper in laboratory and field settings (3, 9) and was shown to provide accurate ferritin measurements. Other studies have used filter paper cards such as Schleicher & Schuell grade 903 (Keene, NH), and the National Committee on Clinical Laboratory standards (20) recommends using this brand of filter paper because its performance and quality are monitored (21) and it may offer lower batch-to-batch variation when different lots of filter paper are used. In a pilot study (n = 30) we found the variation in ferritin values to be small (<3%) when different lots of filter paper (Whatman #1) were used. Furthermore, in another small experiment (n = 60), we found no significant differences between mean ferritin values (P > 0.10) measured with the use of Schleicher & Schuell grade 903 and Whatman #1 filter paper. Moreover, the cost of Schleicher & Schuell grade 903 paper is 3–4 times the cost of Whatman #1 filter paper, which could be an issue when resources are limited. With respect to the use of desiccant to store DSS samples, there are some indications that this practice may offer greater stability for ferritin (4). We used the simple and practical approach of using a locally available desiccant (calcium sulfate) as a precautionary measure and obtained accurate results for serum ferritin with the DSS ferritin methods. Pending further evaluation of the effectiveness of humidity on stability of serum ferritin in very humid settings, we recommend storing DSS samples with a desiccant. Desiccant packs offer a more convenient option and, as an additional security measure, humidity indicator cards can be used until the samples are analyzed (20, 21). Further steps toward making the simplified DSS methods for ferritin determination even more field-user friendly, for use in remote areas without access to electricity, would include the use of battery-operated microcentrifuges and the possible use of gravity sedimentation techniques to separate serum from blood cells.

In conclusion, the results of this field study corroborate our previous findings that the standard DSS ferritin method (ie, DSS A) is valid for the measurement of ferritin. Furthermore, our findings show that simplified techniques for the preparation of DSS samples for ferritin analysis with the DSS ferritin method are promising. In particular, our findings suggest that DSS samples prepared by using techniques such as blotting a large circle of serum onto filter paper (DSS B) or using a commercial dispenser to push serum from a capillary tube (DSS C) and analyzed by using the DSS ferritin method yield mean ferritin values that are not significantly different from those obtained with the traditional method of ferritin measurement. Thus, these methods may be suitable for population surveys because they require less training for field personnel. The use of simplified techniques, such as the DSS C approach, may also be advantageous for the preparation of DSS samples for the measurement of other micronutrients and warrants further investigation. The one-time investment of ≈US$159 for the dispenser could yield a simpler and safer method for the preparation of DSS samples. We recommend the exploration and validation of the practical considerations that have arisen from the Guatemalan experience, while studying populations with a high prevalence of iron deficiency, to confirm and extend the current promising findings concerning the use of the simpler DSS techniques for ferritin measurement.

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NA was responsible for developing the study design. NA and JB established the study protocol and analysis. NWS and EB contributed to the process. JB supervised the data collection and DSS preparation by M-ER-A and MMH. NA supervised the spot ferritin assay. Data analysis was conducted by NA and JB. NA wrote the manuscript. All coauthors contributed to the manuscript development process. None of the authors had a conflict of interest.

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