Screening of folate status with use of dried blood spots on filter paper\textsuperscript{1–4}

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ABSTRACT
Background: Dried blood spots (DBS) on filter paper have been a successful and economical matrix for neonatal screening.
Objective: Our objective was to develop and evaluate an optimized method for DBS folate analysis and to assess DBS folate stability.
Design: DBS were eluted from paper by sonication in 5 g ascorbic acid/L containing 0.1% (by vol) Triton X-100 and hemoglobin folate values (HF; as pmol/g) were calculated from DBS eluate folate and hemoglobin concentrations.
Results: Over 95% of DBS folate was eluted during a standardized sonication cycle and DBS folate assay reproducibility was acceptable both within (CV: ≤ 8%) and between (CV: ≤ 9%) runs. HF means (±1 SD) from finger-stick DBS and conventional venous methods were 2513 ± 1144 and 2607 ± 1195 pmol/g, respectively, in blood samples taken concurrently from 80 donors, and they correlated well ($r = 0.97$, $P < 0.001$). HF values and erythrocyte folate measures may be interconverted by using the mean cell hemoglobin concentration.
Conclusion: The DBS matrix has potential as an inexpensive and practical option for folate screening studies.

INTRODUCTION

Dried blood spots (DBS) prepared from peripheral blood have been a successful matrix for epidemiologic screening. This method, in which finger-stick blood, collected onto special filter paper and then dried and later eluted for testing, has been used for semiquantitative analysis of amino acids (1, 2), hormones (3), lipids (4), and therapeutic drugs (5), as well as for genetic screening (6). The DBS matrix has been used most successfully in neonatal screening for metabolic defects such as phenylketonuria, for which normal and abnormal results are significantly different (7); it has been considered unsuitable for measuring vitamin B-12 and folate concentrations, which require a higher degree of accuracy (8, 9).

We recently described a folate-status screening method in which hemoglobin and folate concentrations (or hemoglobin folate values; HF) are measured in finger-stick peripheral blood samples (10). HF values were calculated by dividing the whole blood–folate concentration by the sample hemoglobin concentration. In preliminary validation studies, HFs correlated well with the erythrocyte folate concentrations of normal Americans measured by conventional methods ($r^2 = 0.99$; $n = 11887$). Significantly, a hemoglobin-folate ratio could be determined accurately in specimens when the precise blood volume or dilution factor was unknown. This approach facilitated accurate folate analysis in small peripheral blood samples and suggested the potential of a DBS folate assay application (10). This study describes the optimization of a DBS folate assay.

MATERIALS AND METHODS

Materials
Sodium ascorbate, ascorbic acid, sodium lauryl sulfate (SLS), sodium dihydrogen orthophosphate, disodium hydrogen phosphate, Tween 80, and Triton X-100 were from Sigma Chemical Co (St Louis). “In-house” single-distilled water was used throughout the analyses. Disposable borosilicate tubes (12 × 75 mm) were from Corning Glassworks (Corning, NY). Cryovials were from Sarstedt (Wexford, Ireland). Venous blood was collected into (6-mL) K\textsubscript{2}-EDTA–containing Vacutainers (Becton Dickinson, Orangeburg, NY), and finger-stick incisions were made with both Microtainer safety-flow lancets (Becton Dickinson, Rutherford, NJ) and Unistik 2 lancets from Owen Mumford Inc (Atlanta). Desiccant packets (silica gel) were from Multiform Desiccants, Inc (Buffalo). Resealable plastic bags were from Fisher Scientific (Pittsburgh). Sonicators, models FS-100 and FS-200b, were from Decon Ultrasonics Ltd (Sussex, United Kingdom); the FS-200b model had a heating element.

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Complete blood counts were obtained with use of a Coulter STKS analyzer (Coulter Electronics, Hialeah, FL). Hemoglobin concentrations were also measured manually with a model U-2000 Hitachi spectrophotometer with a sample-sipper unit (Hitachi Ltd, Tokyo).

Type 903 (lot W921) filter paper cards (15 × 10 cm) with pre-printed (12.5 mm) circles from Schleicher and Schuell (Keene, NH) were used throughout. 5C plus Coulter Counter cell controls (Coulter Diagnostics) and CBC-8 hematology controls (R+D Systems, Minneapolis) were used as hemoglobin controls. Vortex mixing was done with the Vortex Genie-2 (Scientific Industries Inc, Bohemia, NY) and roller mixing was done with the Coulter Mixer (Coulter Electronics, Hertfordshire, United Kingdom).

Folate assays

Folate microbiological assays on microtiter plates were conducted with chloramphenicol-resistant Lactobacillus casei (11), and assay performance was monitored with in-house controls (12). Folate results were calculated as ng/mL and converted to nmol/L or pmol/g (1 nmol/L = 2.265 ng/mL).

Hemoglobin assays

The hemoglobin concentrations of DBS eluates and of whole blood lysates in ascorbic acid were measured spectrophotometrically after dilution in an SLS solution consisting of 0.7 g SLS and 1 mL Triton X-100 in 1 L of 0.033 mol phosphate buffer/L, pH 7.3 (10). Briefly, DBS eluates with ascorbate or whole blood lysates with EDTA (EDTA-WB; 1:19 dilution in 5 g ascorbic acid/L) were diluted 1:10 in SLS solution and allowed to stand for 3 min; hemoglobin concentration was measured at 535 nm within 60 min. Control lysates with low, medium, and high hemoglobin concentrations were included in each run as calibrators and hemoglobin concentrations were calculated automatically by the spectrophotometer software. Hemoglobin control lysates were a 1:19 dilution (in 5 g ascorbic acid/L) of either commercial hemoglobin controls or fresh EDTA-WB lysates, with the hemoglobin value verified by a reference method (13). Hemoglobin control lysates stored in aliquots at −20°C remained spectrophotometrically stable as hemoglobin assay calibrators for ≥6 mo.

Dried blood spots

Finger-stick DBS from volunteer donors were obtained by recommended procedures unless specified otherwise (14). An attempt was made to fill the preprinted 12.5-mm circle on the filter paper cards. Some finger-stick DBS taken from blood bank donors were of poor quality. Nurses attending to blood donors sampled these DBS from unwarmed finger-stick sites just after sampling blood from the same site for hemoglobin screening. For convenience, DBS used in the stability and extraction studies in this paper were venous EDTA-WB samples that were spotted onto paper by pipette in either 100- or 50-µL aliquots. All DBS cards were placed horizontally on a rack and air-dried overnight at ambient temperature unless stated otherwise.

Principle of dried blood spot folate analysis

Folate and hemoglobin from DBS are coeluted into an ascorbate-detergent solution [AATX: 5 g ascorbic acid/L containing 0.1% (by vol) Triton X-100] during a standardized sonication cycle. Hydrolysis of erythrocyte 5-methyltetrahydrofolate poly-glutamates by endogenous DBS plasma γ-glutamyl hydrolase proceeds during elution. DBS eluate folate and hemoglobin concentrations are then assayed by using an assumed common dilution factor (1:20); whole blood folate results (ng/mL or nmol/L) are expressed as HF values (ng/g, nmol/g, or pmol/g) after division by the hemoglobin result (g/L) as follows:

\[
\text{Hemoglobin folate (nmol/g) = WBF (nmol/L) /hemoglobin (g/L)}
\]

HF may thus be determined independently of the DBS blood volume or its dilution factor (10). Whole blood folate concentrations (WBF) are traditionally adjusted to erythrocyte folate concentrations (RCF) by dividing by the sample hematocrit (15); alternatively, they may be adjusted to the HF value because of the constancy of the mean cell hemoglobin concentration (MCHC), which is the ratio of hemoglobin concentration to hematocrit in blood. This approach has been validated in blood samples from sick, hospitalized patients who were folate deficient and also in normal samples (10) in which RCF (nmol/L) and HF values (nmol/g) correlated well \( r^2 = 0.993; n = 11887 \). HF and RCF data may be interconverted (10) by using either specific or population mean MCHC values as follows:

\[
\text{RCF (nmol/L) = [HF (pmol/g) × MCHC (g/L)]/1000}
\]

Extraction optimization

Efficient and consistent extraction of both DBS hemoglobin and DBS folate is essential for DBS folate analysis because the HF is calculated by using both variables. More than 60% of erythrocyte 5-methyltetrahydrofolates are polyglutamates (16); they are converted to assayable folate monoglutamates during exposure to endogenous γ-glutamyl hydrolase at a pH optimum of 4.5 (15). In preliminary DBS elution studies, we recorded the highest overall erythrocyte folate concentrations when sonicating DBS in ascorbic acid solutions with detergent added. This approach allowed endogenous serum (plasma) γ-glutamyl hydrolase activity to proceed at an optimal pH during DBS elution while stabilizing blood folates. Freshly prepared AATX was chosen as a suitable extraction solution.

Dried blood spot preparation

Two methods were used: either entire DBS were cut from the paper card with scissors and then cut in half for extraction, or at least two 6.35-mm DBS punches were removed from each DBS by using a standard paper punch. DBS material was placed into a round-bottomed, disposable borosilicate tube (12 × 75 mm) covered in AATX solution, vortex mixed, and sonicated. DBS eluates were later decanted into a fresh tube. When whole DBS were extracted, the volume of AATX added was roughly equivalent to 20 times the DBS blood volume. Each DBS punch (6.35 mm diameter) was considered to represent ~12.5 μL whole blood on the basis of blood-labeling \( ^{3} \text{H} \text{Cr} \) studies (9), and the extraction of 2 DBS punches in 0.5 mL AATX provided a volume of eluate sufficient for both hemoglobin spectrometry and folate bioassay. Control whole blood samples used to monitor DBS extraction efficiencies were prepared by conventional volumetric dilution of EDTA-WB 1:9 in 10 g ascorbic acid/L (15) and by mixing the solution on a roller mixer for 30 min at 22°C. No significant difference was noted between the folate results of lysates prepared by dilution of EDTA-WB 1:9, 1:19.
or 1:29 in either 10 or 5 g ascorbic acid/L or with the inclusion of 0.1% (by vol) Triton X-100.

**Optimization of dried blood spot elution**

Entire 50-μL DBS prepared from 7 venous EDTA-WB samples as described were extracted in batches by adding 1 mL AATX and immediately sonicating them (model FS-100). Sonication proceeded at ambient temperature and eluates were removed, vortex mixed, and decanted after 5, 10, 15, 20, 30, 45, 55, 75, and 90 min. The eluates were assayed for both hemoglobin and folate concentrations and compared with EDTA-WB control values. Later, a larger sonicator (model FS-200b) was used and elution efficiency was evaluated in a similar manner.

**Supplementation with γ-glutamyl hydrolase**

Additional serum γ-glutamyl hydrolase was added to some DBS extracts as a control for enzyme instability or inactivation in the paper matrix during storage. This supplementary serum γ-glutamyl hydrolase was added as a volume of fresh, unprocessed low-folate (<2 μg/L) human serum equivalent to half the DBS blood volume.

**Comparison of folate concentrations of dried blood spot punches and entire dried blood spots**

DBS were prepared from 20 venous EDTA-WB samples. Control lysates were prepared at the same time by 1:20 dilution in AATX. DBS were extracted in 3 ways: 1) by extraction of the entire DBS (100 μL), 2) by extraction of 6.35-mm DBS center punches, or 3) by extraction of 6.35-mm DBS peripheral punches. DBS punches (6.35 mm) were considered to be roughly equivalent to 12.5 μL whole blood (9).

HF values of entire finger-stick DBS, 6.35-mm DBS punches, and venous controls from 13 volunteer donors were compared. One central and 1 peripheral 6.35-mm DBS punch were extracted together in 500 μL AATX.

**Dried blood spot drying time**

We studied the effect of DBS drying time on DBS folate results. DBS from 5 donor venous EDTA-WB samples were dried at 22°C for 2, 5, 7, 9.5, and 18 h (overnight), and then placed in resealable bags and frozen (−20°C) at the end of each time period. Finally, all DBS were extracted and assayed.

**Precision analysis**

Fourteen 50-μL DBS prepared from each of 4 separate venous EDTA-WB samples were extracted and assayed for hemoglobin and folate in a single assay run. Identical DBS were stored at −20°C in resealable bags with desiccant packets. These DBS were used for between-run quality-control assessments. They were included in weekly routine diagnostic assay runs alongside conventional in-house quality-control materials (12) over 11 wk. The reproducibility of both the DBS folate and hemoglobin assays was determined.

**Comparison of dried blood spot and conventional venous whole blood folate assays**

Venous dried blood spots and venous whole blood samples

Routine diagnostic venous EDTA-WB samples (n = 60) in several batches were used to prepare 50-μL DBS and were diluted at the same time for conventional erythrocyte folate analysis. Both DBS and EDTA-WB lysates were stored frozen at −20°C in resealable plastic bags. They were eventually assayed for folate and hemoglobin and the HF values compared.

**Finger-stick dried blood spots and venous whole blood samples**

Finger-stick DBS were obtained from 80 normal volunteers (in 10 batches) by spotting blood from the finger-stick site directly onto the filter paper card. Venous EDTA-WB sample controls were obtained at the same time and lysates prepared. DBS from laboratory staff volunteers were obtained by using recommended procedures (14). DBS from blood bank donors were of lesser quality, having been taken from unwarmed finger-stick sites secondary to a primary sampling from the same site for hemoglobin screening. We considered DBS to be of poor quality if they were of small volume or smeared. DBS and lysates were stored frozen (−20°C) in resealable plastic bags until assayed for HF values.

**Dried blood spot folate stability**

**Short-term stability**

Fifty-microliter DBS from 15 donor EDTA-WB samples were placed in resealable plastic bags with desiccant on day 1 and stored in the dark at 4°C, 22°C, and 37°C. After 2, 4, 5, and 7 d, the DBS were removed and placed at −20°C until eluted and assayed. A portion of each DBS extract was incubated with additional, fresh low-folate serum. This was done as a control for the possibility of endogenous plasma γ-glutamyl hydrolase instability under some conditions of storage.

**Long-term stability**

Fifty-microliter aliquots from 5 donor EDTA-WB samples were dispensed into cryovials and stored with 50-μL DBS from these same samples in resealable bags at 4°C and 22°C. After 4 d, and 1, 2, 3, and 4 wk, the DBS were extracted and whole blood hemolysates were prepared from the EDTA-WB samples in AATX; these were all stored at −20°C until assayed. A portion of each DBS extract was also incubated with additional fresh, low-folate serum as a control.

**Dried blood spot stability at −20°C**

Finger-stick and EDTA-WB sample DBS along with control lysates with ascorbate (n = 6) from each of 10 donors were placed in resealable plastic bags with desiccant packets and frozen at −20°C. After 12 mo, all samples were removed from storage and assayed; DBS folate concentrations and EDTA-WB lysate folate concentrations were compared. Care was taken not to remove from cold storage, even briefly, DBS that were not to be assayed immediately. The high surface-to-volume ratio of DBS leads to rapid thawing and potential folate instability.

**Statistical analysis**

Statistical analysis was performed by using the statistical packages STATGRAPHICS (version 1.2; Statistical Graphics Corp, Rockville, MD) and STATVIEW (version 4.5; Abacus Concepts Inc, Berkeley, CA). Data are expressed as means ± SDs. Student’s t tests for paired data were used to test whether means were significantly different and significance was set at P = 0.05. Differences between DBS and conventional folate assay results were evaluated by using the methods of Bland and Altman (17) and plotting mean differences against...
means of the 2 methods. Spearman rank correlation coefficients and simple regression analysis were also used to compare data.

RESULTS

Dried blood spot extraction

We considered that elution of erythrocyte folate was probably a function of cell lysis, enzymatic hydrolysis, sonication conditions, and other factors. Folate elution was always slower than hemoglobin elution in typical DBS elution profiles (Figure 1), and DBS elution was slower when using the Decon model FS-100 sonicator (Figure 1A) than when using the more powerful model FS-200b unit (Figure 1B). This illustrates the importance of establishing DBS elution profiles for individual sonicators under standard conditions and adhering to them. Failure to complete a full cycle will result in a falsely diminished folate estimation. We chose a standard cycle of 60 min when using the FS-200b sonicator and noted that extended sonication cycles of ≤3 h under these conditions neither improved folate elution nor induced loss due to folate instability.

Dried blood spot drying time

Air drying of DBS at room temperature for periods ≤20 h did not significantly influence DBS folate concentrations. Folate concentrations were not enhanced by adding supplementary serum γ-glutamyl hydrolase or by freezing DBS (−20°C) before extraction.

Comparison of hemoglobin folate values in dried blood spots and 6.35-mm dried blood spot punches

HF values estimated with control lysates (3102 ± 2139 pmol/g), entire DBS (3043 ± 2175 pmol/g), or with central (3200 ± 2474 pmol/g) or peripheral (3057 ± 2159 pmol/g) 6.35-mm EDTA-WB DBS punches were not significantly different when compared by using Student’s t test for paired data. With finger-stick DBS we noted no significant difference between HF values of entire DBS (2826 ± 2051 pmol/g), 6.35-mm DBS punches (2876 ± 2159 pmol/g), and control venous EDTA-WB lysates (2864 ± 1686 pmol/g) from 13 donors. Processing DBS punches in the laboratory was faster and more practical than processing whole DBS. In addition, punches acted as a useful rough gauge of blood volumes, allowing us to target practical extraction ratios easily and avoid redilution and assay repeats.

Precision

Within-run and between-run quality-control values for the determination of folate and hemoglobin concentrations and HF values are expressed in Tables 1 and 2. The between-run reproducibility CV for mean DBS HF of <8% compared well with that of 4 conventional in-house lysate controls that were stored and assayed in parallel. These had CVs of 7.2%, 4.3%, 7.2%, and 7.8%, respectively.

TABLE 1
Within-assay variation in the recovery of folate and hemoglobin from dried blood spots (DBS)

<table>
<thead>
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<th>DBS sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>112</td>
<td>87.5</td>
<td>94.4</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.5</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>235</td>
<td>230</td>
<td>328</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5.2</td>
<td>8.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Folate/hemoglobin (pmol/g)</td>
<td>2082</td>
<td>2635</td>
<td>3486</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.1</td>
<td>7.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*7; 14 estimations/sample.
Comparison of dried blood spot and venous whole blood HF values

The mean HF values of venous EDTA-WB samples (n = 60), when assayed as conventional control blood samples and after elution from DBS, were 1295 ± 784 and 1255 ± 850 pmol/g, respectively. The limits of agreement (−2 SD to 2 SD) were 299.6 and 278.8 pmol/g (Figure 2). The results correlated well (r = 0.88, P < 0.001). The HF values of venous EDTA-WB samples and finger-stick DBS taken from normal volunteers (n = 80) were 2607 ± 1194.8 and 2513 ± 1144 pmol/g, respectively. The limits of agreement were 2464 and 661 pmol/g (Figure 3B) and the results also correlated significantly (r = 0.97, P < 0.001; Figure 3A).

In early studies, the standard DBS area (circle of 12.5 mm diameter) was a useful rough gauge of blood volume. In exceptional cases with poor-quality DBS, the eluate hemoglobin concentration could be used as a rough guide to a subsequent folate assay dilution. In later studies, standard DBS punching (6.35 mm) was particularly useful when dealing with poor-quality material.

Stability studies

DBS folate stability was temperature- and time-dependent with little loss of microbiologically assayable folate in the short term (Figure 4). Percentages of folate remaining were estimated relative to initial control EDTA-WB and not DBS folate concentrations; they thus represent DBS extraction efficiency as well as folate oxidative degradation. Extended stability studies (Figure 5) confirmed the superiority of the DBS matrix over the EDTA-WB matrix for long-term storage under all conditions. Folate concentrations in both whole blood and DBS were similar for ≤3 d at 22°C, but after 3 wk < 10% of whole blood folate concentrations compared with > 50% of DBS folate concentrations were assayable (Figure 5). DBS hemoglobin had good stability under all conditions with 95% of initial EDTA-WB concentrations retained after 1 wk compared with 92% retained after 4 wk at 22°C. Difficulties encountered in resuspending aged EDTA-WB may have contributed to the diminished folate concentrations, but elution of aged DBS might equally benefit from extended sonication cycles and needs evaluation. DBS serum γ-glutamyl hydrolase stability was not a concern because additional γ-glutamyl hydrolase could be added as fresh serum. In fact, supplementary serum γ-glutamyl hydrolase did not increase DBS folate concentrations significantly, regardless of DBS age or storage conditions, even after storage for ≤4 wk at 37°C. Folate concentrations of individual donor (n = 10) finger-stick DBS and EDTA-WB DBS were 93.5 ± 4.2% and 89.5 ± 5.8% of control.

![FIGURE 2. Difference plot comparing the hemoglobin folate concentrations (folate concentration/sample hemoglobin concentration) of the dried blood spots (DBS) of whole blood samples with EDTA (EDTA-WB) with corresponding EDTA-WB sample controls.](image-url)

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>DBS sample</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>93</td>
<td>118</td>
<td>122</td>
<td>79.2</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.4</td>
<td>2.9</td>
<td>3.9</td>
<td>4.3</td>
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<tr>
<td>Folate (nmol/L)</td>
<td>143</td>
<td>225</td>
<td>271</td>
<td>223</td>
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<tr>
<td>CV (%)</td>
<td>8.1</td>
<td>6.6</td>
<td>8.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Folate/hemoglobin (pmol/g)</td>
<td>1534</td>
<td>1917</td>
<td>2230</td>
<td>2855</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.5</td>
<td>8.5</td>
<td>8.6</td>
<td>7</td>
</tr>
</tbody>
</table>

*Note: reproducibility was calculated over 11 separate weekly assays.*
concentrations when stored at \(-20^\circ C\) for 12 mo. EDTA-WB lysates were again used as the 100% controls; these lysate folate concentrations remain stable for \(\geq 2\) y at \(-20^\circ C\) (12).

**DISCUSSION**

DBS are an established and successful matrix for field-study screening of numerous analytes (18). Conventional DBS methodology can be insufficiently precise for some applications (9) because analytic accuracy is influenced by variables such as paper performance characteristics, blood spot size, blood hematocrit (8, 19), and analyte distribution (9). In this novel DBS assay, folate concentrations are expressed as a ratio to coeluted hemoglobin and analytic precision is consequently independent of the DBS area-volume relation. HF measurement allows accurate folate analysis of blood specimens of unknown volume or dilution that negate hematocrit measurement, such as small finger-stick peripheral blood samples (10) or DBS. HF measure-
FIGURE 4. Effect of temperature of storage on the short-term stability of folate in dried blood spots (n = 15). Columns represent the mean percentage of folate remaining relative to a control; error bars indicate 1 SD.

FIGURE 5. Effect of temperature of storage on the long-term stability of folate in specimens (n = 5) stored as dried blood spots (DBS) and as whole blood samples with EDTA (EDTA-WB). Columns represent the mean percentage of folate remaining relative to a control; error bars indicate 1 SD.
ment also simplifies DBS sampling by negating the stringent control of variables inherent in traditional collection procedures.

Blood folate concentrations are traditionally adjusted by using the erythrocyte volume (hematocrit) and alternative adjustment using hemoglobin relies on the constancy of the hemoglobin-hematocrit ratio, which is the MCHC in g/L (10). The MCHC is physiologically controlled within relatively narrow limits (20) and its suitability for the interconversion of erythrocyte and hemoglobin folate data was validated previously in a study of almost 12000 healthy Americans, as well as in clinical blood samples (10).

DBS extraction by sonication in ascorbic acid solution allowed endogenous serum (plasma) γ-glutamyl hydrolase activity to proceed during elution, yielding optimal assayable folate concentrations (Figure 1). We did not consider a total DBS elution to be essential provided that eluate folate and hemoglobin concentrations were adequate and reproducible. Nor did we seek an absolute agreement between DBS and conventional folate assay results; folate assays differ and must be interpreted by using method-specific normal ranges (21). In fact ~95% of both DBS folate and DBS hemoglobin were recovered after a standardized sonication cycle (Figure 1).

DBS assay reproducibility was also good (Tables 1 and 2) given that DBS quality control monitors both the elution and the assay of folate and hemoglobin whereas conventional quality control represents whole blood folate analysis alone. Finger-stick DBS and venous EDTA-WB folate concentrations of specimens from 80 normal volunteers were similar, illustrating the potential of DBS for conventional applications as well as for field-study screening (Figure 3). Tailoring the DBS matrix for analysis by competitive protein binding folate assays is underway; these assays require larger sample volumes and will need longer eluate incubation periods to facilitate a complete hydrolysis of folate polyglutamates (22).

DBS folate values are by necessity composite values representing both serum and erythrocyte components. They may be influenced by high serum folate peaks arising from recent folate supplementation (23). Consequently, DBS sampling of fasting subjects may be the preferred option in certain folate intervention studies. Alternatively, when accurate concurrent serum folate and hemoglobin (or hematocrit) results are available, the DBS serum concentration may be calculated and then subtracted in the conventional way.

Detailed information on folate stability in DBS was a priority because of potential field-study applications. DBS may be assayed either immediately, after a few days storage at ambient temperatures in resealable bags with desiccant (Figure 4), or after freezing at −20 °C for ≤1 y. At ambient temperatures, >90% of DBS folate was stable for ≤1 wk. This stability is similar to that of conventional EDTA-WB samples, which we use for routine diagnostic screening (10). The superior stability of DBS folate concentrations over those of EDTA-WB samples in a study lasting ≤4 wk (Figure 5) probably relates to the dryness of the matrix. Such extended studies of DBS folate degradation have little relevance to epidemiologic applications that require analytic accuracy. They do, however, evaluate the potential of DBS for monitoring compliance with supplementation in studies involving folate intervention. In these studies, DBS folate concentrations could be compared with cutoff values predetermined by using stored DBS from valid supplemented and nonsupplemented control subjects. These control DBS would have been obtained and stored under preanalytic conditions appropriate to the study.

The fact that 60% of DBS folate activity was retained after 2 wk at 22 °C whereas only 20% of EDTA-WB folate activity remained (Figure 5) shows the potential of the DBS matrix for such applications. The DBS matrix would be an economical storage vehicle when compared with the alternative—expensive dry ice storage and shipment as venous whole blood extracts with ascorbate, which is the current practice. Whole blood extracts are both expensive and difficult to prepare accurately in field settings and have limited stability (10). The DBS matrix minimizes the need for specialized facilities for collecting and processing blood samples. DBS may be collected by less-qualified personnel (14) and are easily stored and transported.

Folate status is now a serious public health issue relevant to the etiologies of neural tube defects (24), vascular disease and thrombosis (25), and some cancers (26). DBS folate analysis may be a practical way of fulfilling the escalating demand for folate blood screening, while retaining the traditional advantages of DBS collection, which are economy and simplicity.

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REFERENCES


