Use of Blood Specimens Collected on Filter Paper in Screening for Abnormal Hemoglobins

Robert M. Schmidt,¹ Effie M. Brosious,¹ Solomon Holland,¹ Jane M. Wright,¹ and Graham R. Serjeant²

Both cellulose acetate electrophoresis and citrate agar electrophoresis were performed on 834 blood samples collected on filter paper in Jamaica and shipped for testing to the National Hemoglobinopathy Standardization Laboratory at the U.S. National Center for Disease Control. Additionally, 30 blood samples collected locally were stored on filter paper, in microhematocrit capillary tubes, and as whole blood specimens; at selected times the samples were tested for stability to determine the best sample-collection technique for hemoglobin electrophoresis. Results were most nearly accurate when both cellulose acetate electrophoresis and citrate agar testing were used. The methods are easy to perform, but results are unreliable if the blood samples on filter paper are stored at 4 °C for longer than two weeks before they are tested.

Additional Keyphrases: hemoglobinopathies • sickle cell anemia • electrophoresis

Several recent reports have suggested that blood specimens collected on filter paper for hemoglobinopathy testing are quite suitable for mass-screening programs (1–3). In addition, Garrick et al. (4) suggested that specimens collected on filter paper can be used to detect sickle cell anemia and to identify other hemoglobinopathies in newborns. Although the work of these investigators indicated that this is a reliable, efficient method of detecting abnormal hemoglobins, their results were not definitive because their 2435 cord-blood specimens included no FS phenotypes and only 14 FAS phenotypes. If blood samples collected on filter paper from a finger prick can in fact be stored and shipped to a central laboratory, this method of collection would be particularly useful for hemoglobinopathy screening programs. Our study was designed to investigate whether accurate results can be obtained with such samples.

Materials and Methods

Cord-blood specimens from 834 consecutive deliveries at the Royal Victoria Jubilee Hospital in Kingston, Jamaica, were collected in heparinized tubes and on filter paper. Staff of the Medical Research Council Epidemiology Unit, University of the West Indies, screened the blood specimens collected in tubes for abnormal hemoglobins by the methods described by Serjeant et al. (5). The corresponding filter paper samples were stored at 4 °C for two weeks during the entire collection/screening procedure and then were air-mailed in refrigerated containers to the National Hemoglobinopathy Standardization Laboratory located at the Center for Disease Control.

When the specimens arrived at the Atlanta laboratory, 0.6-cm (diameter) discs of filter paper containing the blood spots were punched out and placed in labeled test tubes. About 0.2 ml of a tetrasodium ethylenediaminetetraacetate (EDTA) solution, 2.5 g/liter, was added to each tube to elute the hemoglobin from the paper discs. The tubes were left overnight at 4 °C. The next day, cellulose acetate electrophoresis of the eluted hemoglobin was performed as described previously (6). Subsequently, citrate agar electrophoresis was also done on the eluted hemoglobin samples by the method of Milner and Gooden (7). All specimens were tested in Atlanta no longer than four weeks after the initial testing in Jamaica.

We recommend that either the Beckman Reagent Pack or the laboratory-prepared reagent (with use of Worthington urease) be used with the Beckman BUN Analyzer.

References


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Table 1. Phenotypes of 834 Cord Blood Samples as Determined by Three Methods

<table>
<thead>
<tr>
<th>Electrophoretic method and sample</th>
<th>Total no. specs.</th>
<th>FA</th>
<th>FAS</th>
<th>FAC</th>
<th>FS</th>
<th>FA Bart’s</th>
<th>FAS Bart’s</th>
<th>Homozygous Hb A%</th>
<th>FS Bart’s</th>
<th>F-Victor Jubilee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate screen (both cellulose acetate &amp; citrate agar)</td>
<td>834</td>
<td>699</td>
<td>76</td>
<td>26</td>
<td>3</td>
<td>24</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cellulose acetate on mailed filter paper specimens</td>
<td>834</td>
<td>769</td>
<td>32</td>
<td>22</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cellulose acetate &amp; citrate agar on mailed specimens</td>
<td>834</td>
<td>728</td>
<td>70a</td>
<td>25</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

a One sample here was a true FA or normal specimen, but was categorized as FAS because of the extreme electrophoretic smearing.

Table 2. Errors Made in Phenotyping 834 Cord Blood Samples Mailed on Filter Paper and Tested by Electrophoresis on Cellulose Acetate and Citrate Agar

<table>
<thead>
<tr>
<th>No. false positives</th>
<th>FAS</th>
<th>FAC</th>
<th>FA Bart’s</th>
<th>FAS Bart’s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. false negatives</td>
<td>5</td>
<td>1</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

In addition, 10 normal and 20 abnormal specimens of whole blood were obtained from a local Atlanta hospital. Within 4 h of blood collection each of the 30 specimens was divided for storage into three aliquots. One portion was stored in an evacuated blood-collection tube containing Na2EDTA, one was stored in a heparinized microhematocrit tube, and the third was spotted on filter paper. Half of each of the three aliquots was kept at room temperature for 30 days, the other half in the refrigerator for 30 days. On days 3, 7, 14, 20, and 30, cellulose acetate electrophoresis and solubility tests were run on one sample from each aliquot. The electrophoresis was graded as to the amount of smearing present and as to whether discernible bands could be seen in the areas where bands had been found in initial testing.

Results

Table 1 lists the phenotypes assessed in Atlanta with the cellulose acetate electrophoresis and citrate agar electrophoresis methods and the phenotypes assessed in Jamaica. Separations on cellulose acetate strips were generally poor, with much streaking and smearing of hemoglobin. Citrate agar patterns showed more definite bands, but substantial smearing between bands persisted. Over 57% of the sickle cell traits and 15% of the hemoglobin C traits were missed when cellulose acetate alone was used. Only 9.2% of the hemoglobin S traits and 4% of the hemoglobin C traits were missed when the combination of methods was used. The citrate agar procedure was needed to detect the hemoglobin S in almost half of the known FAS samples sent. The cellulose acetate procedure was virtually useless for detecting small amounts of hemoglobin. Hemoglobin Bart’s, known to be relatively unstable, was not seen in most instances in the patterns obtained with the mailed filter-paper specimens. Table 2 summarizes these findings as the number of false-positive and false-negative results obtained. Results from part two of this study indicate that, if kept refrigerated, venous blood stored in evacuated blood-collection tubes can be tested satisfactorily after storage for longer than a month; in contrast, samples collected in heparinized microhematocrit tubes and on filter paper can be used satisfactorily for only two weeks. Because the problem of smearing increases with time, specimens stored on filter paper for longer than two weeks are not suitable for testing.

Discussion

The detection of abnormal hemoglobins at birth is important because it enables the physician to begin appropriate preventive-medicine procedures in babies with sickle cell disease, including hydration and early treatment of infections (8). Because hemoglobins S, C, D, and G and other β-chain variants are found only in small concentrations in the blood of newborns, it is generally believed that these variants cannot be detected until the concentration of fetal hemoglobin decreases. However, several investigators have independently reported success in detecting abnormal hemoglobins in cord blood (5, 9–12).

Blood samples collected on filter paper are now being obtained from newborns in 43 states for phenylketonuria screening (13), and this method also appears to be practical for obtaining samples for an abnormal hemoglobin screen. However, storage time and conditions must be considered if this technique is used. The current study indicates that storage of such samples for longer than two weeks results in smearing of hemoglobins, which produces major errors in interpretation of electrophoretic patterns.

References


**Corrections**

**Volume 21**

p 1668: In Figure 2, as shown, R2 and C2 are connected to the ac side of the bridge rectifier. The correct schematic would show the upper end of R2 connected to the anode of the SCR and the lower end of C2 to the cathode of the SCR.

p 1905: Figures corresponding to legends for Figures 3A and 3B are reversed, i.e., 3A is a negative sample, 3B a positive sample.

**Volume 22**

p 102: In both Tables 1 and 2, headings "630 nm" and "410 nm" should be reversed; caption under Fig. 1 should end ". . . solution No. 2."

p 156: Tenth line of last paragraph: change "100 ml" to "100 mol."

p 219, Table 1, fourth item in fourth column: "±68.8" should read "±8.8."


p 273, under Reagents: "ferric nitrate (68.5 g/liter)" should read "mercuric nitrate (68.5 g/liter)"; "mercuric nitrate (202 g/liter)" should read "ferric nitrate (202 g/liter)." In Figure 1 (p 274), 620 μg, 530 μg, and 440 μg should be changed to 62 mg, 53 mg, and 44 mg.

p 344: under Specificity, "1.0 mg/liter" should read "100 mg/liter."

p 379: under Procainamide assay, sentence should be changed to read "Place the containers in an ice bath, add 2.0 ml of hydrochloric acid and 1.0 ml of sodium nitrite, mix the contents. . . . "

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