of the high concentration of bilirubin used.

Results were consistent for more than 50 different sera.

We thank the technicians on our service for their collaboration in this work. Detailed data may be obtained from the editors or the Editorial Office of this journal.

References

Constantino G. Bao1,2
Maria T. Dopico1
Sofía A. Geijo1
Agustín C. Sánchez1
Francisco A. Menendez1,2
1 Servicio de Anál. Clín.
Residencia Sanitaria San Agustín Aules, Oviedo, Spain
2 Dept. de Bioquímica
Facultad de Med.
Universidad de Oviedo, Spain

Rapid Detection and Estimation of Hemoglobin by Agarose Gel Electrophoresis

To the Editor:

It is often necessary to detect hemoglobin F, to diagnose thalassemia. Electrophoresis on agarose gel gives better separations of hemoglobins (J). We use a discontinuous buffer system (Tris–EDTA–boric acid, pH 8.1, for gel; barbitur buffer, pH 8.6, for the electrophoretic tank). Blood was sampled from normal human adults and human umbilical cords into EDTA-containing tubes. Hemolysates were prepared according to Drabkin (2). The concentrations of the hemolysates were adjusted to about 10% and 1%. Gel slides, 2.5 × 7.5 cm or 5 × 7.5 cm, were prepared with 6 g/L agarose (Koch-Light) in Tris buffer. Two to four microlitres of the 1% hemolysate was applied to the gel. Electrophoresis was at 5 mA per microscope slide for 45 min. The hemoglobin bands in the agarose gel were fixed by placing the gel in trichloroacetic acid (100 g/L). The gel was transferred to saline (9 g/L) for about 2 to 3 min, to separate it from the glass slide, and then to filter paper (Whatman No. 1). When it had dried, it was stained with bromphenol blue. The stained hemoglobin bands were cut out, eluted with 0.1 mol/L NaOH, and the color in the eluate was measured at 590 nm. From this, the concentrations of different hemoglobin bands were estimated. The concentration of hemoglobin F was also estimated, for comparison, by the routinely accepted 1-min alkali denaturation method (3), with use of the 10% hemolsate.

This method gives good separations of HbA, HbF, HbS, and HbA2 (Figure 1). Results for HbF by our method were comparable with results by the alkali denaturation method. This simple, rapid method can be easily carried out in the routine laboratory.

References

S. K. Ahaley
M. D. Hegiste
Miraj Medical College
Miraj 416 410, India

Table 1. Change In Measured Glucose In Control Sera After Exposure To Glucose Oxidase Strips *

<table>
<thead>
<tr>
<th>Time after exposure, min</th>
<th>Measured decrease, mg/L</th>
<th>0-2</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>One strip</td>
<td></td>
<td>0</td>
<td>1.8</td>
<td>4.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Three strips</td>
<td></td>
<td>8</td>
<td>40</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

* Duplicate aliquots of pooled human serum were exposed by dipping one or three glucose oxidase test strips into the sera for 60 s. Other aliquots were not exposed. All sera subsequently were allowed to stand, covered, at room temperature for the listed times before glucose analysis in an AutoAnalyzer II (Technicon Corp., Tarrytown, NY 10591) by the method of Trinder (3). * One SD = 27.5 mg/L.

Misuse Of Glucose Oxidase Test Strips

To the Editor:

Glucose oxidase test strips for blood glucose have proven convenient as an aid to immediate decisions in the management of the diabetic patient. In many cases, venous blood is drawn and tested immediately with the test strip, and the rest of the specimen is submitted to the laboratory for a confirmatory glucose determination, and often other analyses as well. Several test-strip preparations are available for use with whole blood and at least one is designed for use with either serum or blood (1). In addition, test strips designed for use with blood have been used, with or without modification, with plasma, serum, or cerebrospinal fluid (2–4). Thus one can use either whole blood or serum for rapid evaluation of glucose before processing for additional analyses.

Recently, we discovered that some attending physicians had adopted the practice of dipping the test strip into the sample for 60 s rather than removing a drop to place on the strip as stated in the manufacturer’s instructions. Because of the potential artefacts introduced in the remaining sample by this procedure, we evaluated glucose in control sera at various times after exposure for 60 s to two commonly used glucose oxidase test strips (B–G Chemstrip from BMC Biodynamics, Indianapolis, IN 46250, and Dextrostix from Ames Co., Miles Laboratories, Inc., Elkhart, IN 46515). The procedure resulted in small but significant decreases in glucose values, which became larger with time after exposure, consistent with elution of glucose oxidase (EC 1.1.3.4) from the test strip into the sample. There was a definite, although nonlinear, dose response (Table 1).

In sixteen additional trials carried out by exposing 2.5 mL of serum aliquots to one strip each for 60 s, with glucose measurement at 120 min, the range of decrease found was 90 to 50 mg/L (mean 57, SD 17.5 mg/L).

During these measurements, we noted that treated sera changed color, from yellow to pale green, about 20 min after exposure. Anticipating additional changes, we performed SMA-12 and -6 profiles on samples of both serum and whole blood after exposure to glucose.