risks of capillary collection are less than that with venipuncture.

In conclusion, amounts of theophylline in serum were determined by standard venipuncture and finger lancet in 20 asthmatic children on maintenance theophylline. By paired t-test we found no difference in the theophylline concentrations from these two methods. Patients' preference was divided between the two methods of collection, but favored venous collection. We thank Marie C. Ragni for her technical assistance and Sumner J. Yaffe for helpful discussion. This research project was supported by NIH Grant HD 10053-02.

References
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Modification of the DuPont aca as Exemplified by a New Procedure for Neonatal Total Bilirubin

Albert D. Fraser and Anita L. Lindsay

Hemoglobin interferes with determination of total bilirubin by the diazo procedure with the DuPont aca. We compared results by that procedure with those by a manual direct spectrophotometric two-wavelength method. Because the manual method was not inhibited by hemolysis as was the DuPont aca diazo procedure, we reprogrammed our DuPont aca to measure the absorbance of diluted serum samples at 452 and 577 nm with use of DuPont absorbance packs as the sample pack and cuvette. Results correlated well with those by the manual spectrophotometric method, and hemolysis no longer interfered. The method is primarily intended for use with serum from neonates. Additional Keyphrases: manual method compared spectrophotometry discrete analyzer With the DuPont aca discrete analyzer, total bilirubin is determined by a modified diazo procedure (1) with the surfactant Tween-20 being used to solubilize unconjugated bilirubin. Blijenberg et al. (2) found that the degree of interference by hemoglobin in the aca bilirubin method was unacceptable, but became constant at high hemoglobin concentrations in plasma. Their solution was to construct a new calibration graph after adding hemoglobin to the buffer diluent to give a final concentration of about 2.42 g of hemoglobin per liter in the test pack. We believed that a better approach for neonatal samples would be to determine bilirubin directly, with the aca being used as a spectrophotometer for determining bilirubin on the basis of its differential absorbance at 452 and 577 nm. Blank absorbance packs were used as the sample carrier in our procedure. The results correlated well with those by the corresponding manual two-wavelength procedure. The new procedure requires less sample than does the diazo method used in the aca and it is not affected by hemolysis.

Materials and Methods

Instrumentation
We used a Model PM2DL spectrophotometer (Carl Zeiss Canada Ltd., Montreal, Quebec) for the manual bilirubin determination. A 20-µL serum sample was diluted with 2.0 mL of phosphate buffer and the absorbance measured at 450 and 575 nm as originally described by White et al. (3) Total bilirubin was measured with an aca II (DuPont Co., Instrument Products, Automatic Clinical Analysis Division, Wilmington, DE 19898). We evaluated their diazo procedure (1) and also used their absorbance packs in our two-wavelength direct method adapted to the aca. The following changes were considered optimal for our method: Buffer in position 6, sample volume 40 µL, filter number 5—452 nm, filter number 10—577 nm, theoretical scale factor 0.1803,
theoretical starting point 000.0 The letter wheels were pro-
grammed to print PTBIL (pediatric total bilirubin), second
decimal position. DuPont absorbance packs were used in our
method with the black bar in the first position removed
(method code 62).

Reagents and Procedures

Phosphate buffer (pH 7.4). Dissolve 7.65 g of disodium
hydrogen phosphate and 1.74 g of potassium dihydrogen
phosphate in 1 L of distilled water.

Prepare the standard bilirubin solutions (crystalline biliru-
bin obtained from Fisher Chemical Co., Dartmouth, N.S.)
in bovine serum albumin, according to established procedures
(4).

Prepare hemolysates from fresh human blood by washing
the erythrocytes with cold physiological saline and then lysing
the cells with distilled water. Remove cell stromata by cen-
trifugation and determine the hemoglobin concentration in
the resulting solution by the cyanomethemoglobin procedure
(we used a Coulter Model S, Coulter Electronics, Inc., Hialeah,
FL 33010).

We made dilutions with an albumin/bilirubin diluent to
provide a series of solutions with various hemoglobin con-
centrations but equivalent bilirubin concentrations and an-
alyzed these solutions for bilirubin by all three methods de-
scribed in the Instrumentation section.

We measured plasma hemoglobin according to Hunter
(5).

The reference serum we used was “Versatol Pediatric
Reference Serum” (General Diagnostics, Scarborough, On-
tario).

Sample Collection

All plasma samples were collected by heel prick, into Dade
Natelson Blood Collecting Tubes with ammonium heparin
as anticoagulant (Canadian Laboratory Supplies, Dartmouth,
N.S.).

Results

Linearity. We evaluated the linearity of the direct PTBIL
procedure by use of eight standard bilirubin solutions, in
concentrations ranging from 50 to 400 mg/L, in 50-mg/L in-
crements. The upper limit of linearity for bilirubin was 300
mg/L.

Precision. The results are shown in Table 1.

Analytical recovery. For the PTBIL method, bilirubin rec-
covery was evaluated by assaying solutions of pure bilirubin
in pooled serum. Solutions with five different bilirubin con-
centrations were obtained by diluting the 300 mg/L standard
solution. In this manner we assayed six samples, with con-
centrations ranging from 50–300 mg/L. The mean percentage
recovery was 93% (range 92–94%).

Interferences. Because hemoglobin has been shown to in-
hibit diazo procedures for bilirubin, and because hemoyzied
samples are commonplace when blood is collected from neo-
lates by capillary tubes, we studied the effect of hemolysis on
our bilirubin method(s).

Control serum was supplemented with hemoglobin to give
five concentrations, 250 to 2000 mg/L. Figure 1 shows the in-
hibitory effect of hemoglobin, even at 250 mg/L, on the Du-
Pont acu diazo procedure. In contrast, inhibition by hemo-
globin was not considered significant for the manual and
PTBIL differential spectrophotometric methods.

Split-sample comparison. Plasma samples were analyzed
by the manual two-wavelength method and by our modified
acu method, simultaneously, to avoid any errors caused by the
instability of bilirubin on standing. Bilirubin concentrations
ranged from 29 to 174 mg/L. The results were evaluated by
least-squares linear regression. Comparing the DuPont PTBIL
method (y) with the manual procedure (x) for 56 samples, we
obtained a correlation coefficient of 0.98, with a slope of 0.97
and intercept of 0.3 mg of bilirubin per liter.

Discussion

The manufacturer and others (2) caution the operator of the
acu about using the DuPont diazo procedure when neo-
natal samples are to be analyzed for bilirubin. Blijenberg et
al. (2) measured hemoglobin in 159 neonatal specimens and
reported an average hemoglobin concentration of 1700 mg/L.
From Figure 1 one can see that this would correspond to about
a 25% decrease in the value for apparent bilirubin. One could
use, with some validity, the DuPont diazo procedure to de-
terminate bilirubin in the serum of neonates if the hemoglobin
concentration was < 400 mg/L, but some other procedure not
subject to hemoglobin interference would be more ap-
propriate.

Results by the manual differential spectrophotometric
procedure and our adaption of it to the acu correlate well; they
are unaffected by the concentrations of hemoglobin commonly
found in neonatal samples (6). That the accuracy of our
method was good was indicated by the average recovery ex-
ceeding 93%. The limits of linearity (300 mg/L) were accept-
able, because the method is only intended for use with samples
from neonates.

Lipemia may interfere in our method for bilirubin, but li-

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**Table 1. Precision Data**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean, mg/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within-run</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16.0</td>
<td>0.40</td>
</tr>
<tr>
<td>Abnormal</td>
<td>167.2</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Day-to-day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control sera (lot 1)*</td>
<td>218.1</td>
<td>0.55</td>
</tr>
<tr>
<td>Control sera (lot 2)</td>
<td>210.5</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* Lots 1 and 2 had different lot numbers.

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Fig. 1. Effect of hemolysis on bilirubin determinations

- Manual spectrophotometric, ▲ direct DuPont acu, ● diazo DuPont acu
pemia is not a problem with most samples from neonates, so this interference was not well investigated.

The manufacturer has informed us since our work was completed that they intend to introduce a "neonatal bilirubin" procedure for the aca. The price for the DuPont pediatric test pack is 12% higher than that of absorbance packs we used in our procedure. The saving would be significant with our volume of neonatal bilirubin requests (50-60/week).

We believe that our procedure is reliable for measuring bilirubin in serum of neonates. The method is worthy of consideration by aca users who have to quantitate neonatal samples for bilirubin and do not have a commercial bilirubinometer in their laboratory.

In conclusion, we believe that our work on bilirubin demonstrates the feasibility of users making improvements on the aca to meet the needs of their laboratory.

In a previous publication, we described a procedure suitable for stat determinations of serum digoxin with a commercially available radioassay kit (1). In that emergency procedure, we shortened the incubation time by increasing the concentration of antigen and antibody without altering their absolute quantities in the incubation mixture and by increasing the incubation temperature. These modifications were possible because antibody and radiolabeled antigen were supplied in concentrated solutions.

The kit manufacturer has recently changed the assay protocol. The changes included predilution of the radiolabeled antigen with buffer, which made our previously described emergency procedure inapplicable to this kit. The kit manufacturer suggested a stat procedure that involves doubling the amount of antibody used in the regular procedure to compensate for the decrease in binding that results from use of a shorter (10 min) incubation. This has two obvious disadvantages: increased material cost and decreased sensitivity because of the increase in the binding capacity of the assay. In our modified procedure, we kept the binding capacity constant by decreasing the volume of the incubation mixture without increasing the amount of antibody. Total binding and the rate of binding on 10-min incubation are 50.4 and <1.3% per minute, respectively. Results by our modified stat procedure compare well with those by the manufacturer's suggested regular procedure in terms of sensitivity, accuracy, and precision, and are more sensitive and economical than those by the manufacturer's suggested stat procedure.

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A Commercial Digoxin Radioassay Modified for Use as an Emergency ("Stat") Procedure

I-Wen Chen, Matthew Sperling, and Harry R. Maxon

A recent "stat" procedure recommended by the manufacturer for use with a commercial digoxin radioassay involves doubling the amount of antibody used in the regular procedure, to compensate for the decrease in binding that results from use of a shorter (10 min) incubation. This has two obvious disadvantages: increased material cost and decreased sensitivity because of the increase in the binding capacity of the assay. In our modified procedure, we kept the binding capacity constant by decreasing the volume of the incubation mixture without increasing the amount of antibody. Total binding and the rate of binding on 10-min incubation are 50.4 and <1.3% per minute, respectively. Results by our modified stat procedure compare well with those by the manufacturer's suggested regular procedure in terms of sensitivity, accuracy, and precision, and are more sensitive and economical than those by the manufacturer's suggested stat procedure.

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We describe a more sensitive and more economical stat procedure for digoxin assays with use of prediluted labeled antigen.

Materials and Methods

Materials

The digoxin radioassay kit we used was obtained from Becton Dickinson and Co., Orangeburg, NY 10962. It included a set of digoxin standards, a rabbit antiserum solution in phosphate-buffered saline (10 mmol/L potassium phosphate, 150 mmol/L NaCl, bovine albumin, and preservatives, pH 7.4), a radiolabeled antigen solution containing 3-O-succinyl digoxigenin [125I]l-tyrosine, about 300 pmol (40 μCi) per liter, in phosphate-buffered saline (100 mmol/L potassium phosphate, 150 mmol/L NaCl, and preservatives, pH 7.0), and

References


Eugene L. Seenger Radioisotope Laboratory, Department of Radiology, University of Cincinnati, College of Medicine, Cincinnati, OH 45267.

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