References

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Negative interference with the Du Pont aca Method for Measuring Digoxin—A Reply

To the Editor:

In response to a recent Letter (1) concerning the digoxin method in the aca Discrete Clinical Analyzer, the Du Pont Company would like to offer the following.

As in any enzyme immunoassay, the possibility exists that the enzyme could be either activated or inhibited by drugs and (or) nonspecific substances, resulting in reported values that do not reflect true concentrations of the drug.

We have had occasional reports that samples from patients receiving digoxin gave results of <0.2 ng/mL while reading positive by an alternative method. Taking into account the number of incidents reported to us during the past several years, we judge the frequency of this occurring in the general testing population to be <0.00004.

As a precaution to our customers, we have previously informed them of the potential for this situation to arise.

When methods for digoxin measurement are compared, it is often difficult to establish their accuracy and, therefore, difficult to interpret the findings. This is especially true of the data presented in the recent Letter, which illustrates the inherent problems with interpretation of isolated method-comparison data for digoxin. No method is entirely free from NSIs interference (2).

In fact, according to the authors, three of the four samples were derived from patients having pre-dialysis creatinine concentrations in excess of 50 mg/L—i.e., they are from a population of patients likely to show NSIs interference (3). Thus an equally likely explanation for the results is that none of the methods are giving accurate results. The "true" answer may lie somewhere in between the two and to alert aca analyzer users that the Du Pont method may give "inappropriately low results" is no more warranted than alerting users of the alternative methods that they may give inappropriately high results.

Users of the aca have indicated to us that the Letter implies that the Du Pont method has a general problem with low recovery. The data we have generated in a number of external method correlations totally refutes this implication. Over the past several years, Du Pont has commissioned a number of evaluations in which our method was compared with the Abbott TDX and others. The following tabulation is a representative accounting of these data.

It is clear from these data that the Du Pont method does not show any significant bias or scatter with respect to other commercial methods.

References
2. Skogen WF, Rea M, Valdes R. Endogenous digoxin-like immunoreactive factors eliminated from serum samples by hydrophobic silica-gel extraction and enzyme immunoassay. Ibid., 401–4.

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Choriogonadotropin Interference in a Sensitive Assay for Thyrotropin

To the Editor:

The availability of ultra-sensitive assays for thyrotropin (TSH) prompts investigation of new aspects of sensitivity when assessing thyroid hormone status. Thyrotropin assays have been assessed for potential interferences from other peptide hormones such as lutropin, follitropin, and choriogonadotropin (hCG), and high concentrations of circulating hCG reportedly interfere with the Boots Celltech Sucrose IRMA-TSH (1). Kreutzer et al. (1) added hCG to give a final concentration of 2 × 10^6 int. units/L in the Boots Celltech assay and observed a decrease in TSH from 5.8 to 1.9 milli-units/L. They concluded that this interference by hCG in quantities encountered in early pregnancy is unacceptable.

We have been using the Boots Celltech Sucrose IRMA-TSH for about 18 months. Although we had determined that TSH concentrations in serum of euthyroid women during pregnancy remained within the reference interval (2), we investigated hCG interference in more detail. We collected four pools of plasma containing 8.0, 4.5, 1.0, and 0.6 milli-int. units of TSH per liter and to these we added increasing concentrations of hCG (1st. IRP) ranging from 0 to 2.2 × 10^6 int. units/L and reassayed. The results (Table 1) clearly demonstrate that hCG concentrations such as are encountered during the first two to three months of pregnancy (up to 300 000 int. units of hCG per liter) depress TSH values by 29 to 40%. We stress that in cases of hypothyroidism or noncompliance with prescribed therapeutic agents, represented by a TSH value of 8.0 (normal reference interval 0.4 to 4.5 milli-int. units/L), hCG concentrations such as those seen in early pregnancy would diminish apparent TSH to a value that is still abnormal. Likewise, other pools containing normal TSH concentrations of 4.5 and 1.0 milli-int. units/L were not suppressed to abnormally low values when hCG in concentrations encountered during the first and second tri-

<table>
<thead>
<tr>
<th>Table 1. The Effect of hCG on TSH Concentrations (milli-int. units/L) in Pooled Serum</th>
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</thead>
<tbody>
<tr>
<td>Pool 1</td>
</tr>
<tr>
<td>Mean TSH</td>
</tr>
<tr>
<td>8.0</td>
</tr>
<tr>
<td>4.5</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>0.6</td>
</tr>
</tbody>
</table>

Mean TSH

After addition of 300 000 int. units of hCG

Mean TSH: 5.7, 2.7, 0.6, 0.4

Decrease, %: 29, 40, 40, 33

After addition of 2.2 × 10^6 int. units of hCG

Mean TSH: 2.3, 1.0, 0.15, 0.15

Decrease, %: 71, 78, 85, 75

In the Boots Celltech assay and observed a decrease in TSH from 5.8 to 1.9 milli-units/L. They concluded that this interference by hCG in quantities encountered in early pregnancy is unacceptable.

We have been using the Boots Celltech Sucrose IRMA-TSH for about 18 months. Although we had determined that TSH concentrations in serum of euthyroid women during pregnancy remained within the reference interval (2), we investigated hCG interference in more detail. We collected four pools of plasma containing 8.0, 4.5, 1.0, and 0.6 milli-int. units of TSH per liter and to these we added increasing concentrations of hCG (1st. IRP) ranging from 0 to 2.2 × 10^6 int. units/L and reassayed. The results (Table 1) clearly demonstrate that hCG concentrations such as are encountered during the first two to three months of pregnancy (up to 300 000 int. units of hCG per liter) depress TSH values by 29 to 40%. We stress that in cases of hypothyroidism or noncompliance with prescribed therapeutic agents, represented by a TSH value of 8.0 (normal reference interval 0.4 to 4.5 milli-int. units/L), hCG concentrations such as those seen in early pregnancy would diminish apparent TSH to a value that is still abnormal. Likewise, other pools containing normal TSH concentrations of 4.5 and 1.0 milli-int. units/L were not suppressed to abnormally low values when hCG in concentrations encountered during the first and second tri-

<table>
<thead>
<tr>
<th>Reference method</th>
<th>n</th>
<th>Slope</th>
<th>y-intercept</th>
<th>r</th>
<th>S_\text{xy}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Assays RIA</td>
<td>106</td>
<td>1.050</td>
<td>-0.06</td>
<td>0.950</td>
<td>0.29</td>
</tr>
<tr>
<td>Diagnostic Products RIA</td>
<td>100</td>
<td>1.065</td>
<td>-0.05</td>
<td>0.947</td>
<td>0.20</td>
</tr>
<tr>
<td>Abbott TDX (Version 1)</td>
<td>107</td>
<td>1.047</td>
<td>-0.08</td>
<td>0.974</td>
<td>0.21</td>
</tr>
<tr>
<td>Abbott TDX (Version 1)</td>
<td>89</td>
<td>0.999</td>
<td>+0.03</td>
<td>0.968</td>
<td>0.15</td>
</tr>
<tr>
<td>Abbott TDX (Version 1)</td>
<td>96</td>
<td>1.040</td>
<td>+0.20</td>
<td>0.970</td>
<td>0.19</td>
</tr>
<tr>
<td>Abbott TDX (Version 1)</td>
<td>45</td>
<td>0.980</td>
<td>+0.20</td>
<td>0.970</td>
<td>-</td>
</tr>
</tbody>
</table>
mesters of pregnancy was added.

TSH values during pregnancy are lower when measured by immunoradiometric assay (3); that being the case, a TSH value of 0.6 milli-int. unit/L in such a system is not supposed to an abnormally low value for TSH when 300 000 int. units of hCG are added per liter. In fact, only at concentrations exceeding 1.1 × 10^6 int. units/L did we find suppression to abnormally low values in this study. At hCG concentrations of 2.2 × 10^6 int. units/L the range in decrease of TSH was 71 to 85%.

We think it unwise for clinical chemists to reject TSH assays after assessing interference from hCG by adding such massive concentrations as 2 × 10^6 int. units of hCG per liter. This approach subjects what is potentially a useful tool in thyroid-status studies to situations rarely encountered physiologically. Instead, we would direct attention to a recent study by Klee and Hay (4), who, in an extensive study based on a survey of published studies on sensitive assays for TSH and their own patients, reported that the Boots Celltech IRMA-TSH reagents met all the necessary performance criteria.

References
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dsays of thyrotropin, including effects of varia-
2. McBride JH, Thibeault RV, Rodger-
3. Crow SM, Kellet HA, Seith J, Swee-
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tive thyrotropin assays for an expanded role in thyroid function testing: proposed crite-

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The "Chromogen Oxidase" Activity of Bilirubin Oxidase

To the Editor:

Bilirubin oxidase (EC 1.3.3.5) can be used to remove bilirubin interference from hydrogen peroxide/peroxidase (EC 1.11.1.7) detecting systems (1). One drawback to its use is its inherent "chromogen oxidase" activity, which can lead to large reagent blank absorbances. This "chromogen oxidase" activity can be inhibited with azide or cyanide (2), but this requires incubation of the sample with bilirubin oxidase before assay. This would not be necessary if a chromogen that was not a substrate for bilirubin oxidase could be found. We investigated several phenol derivatives in an attempt to find one that is a substrate for peroxidase but not for bilirubin oxidase.

We added bilirubin oxidase (10 µL of 55 U/L solution in Tris/HCl, 0.2 mol/L, pH 8.4) at 37 °C to 180 µL of various substituted phenols (3 mmol/L in the Tris/HCl buffer also containing 4-
amino-phenazone, 1.0 mmol/L). Absorbance at 510 nm was measured every 20 s for 10 min in a Cobas Bio (Hoff-
mann-La Roche) centrifugal analyzer. To determine the relative absorptivities of the quinoneimine dyes, we used them in the measurement of glucose with glucose oxidase and peroxidase and compared the results obtained with each.

All of the substituted phenols we examined were substrates for bilirubin oxidase (Table 1). The differences between the phenols could largely be explained by the differences in the ab-
sorptivities of the quinoneimine dyes produced (Table 1). Those phenol deri-
vatives that produced a more highly colored dye also produced more color with bilirubin oxidase. Thus, use of bilirubin oxidase to remove bilirubin interference in hydrogen peroxide/per-
oxidase detecting systems, irrespective of which phenol derivative is used, evidently will require prior incubation of sample with enzyme and addition of azide to the assay reagent.

Table 1. "Chromogen Oxidase" Activity of Bilirubin Oxidase on Some Substituted Phenols

<table>
<thead>
<tr>
<th>Phenol derivative</th>
<th>Relative absorptivity of quinoneimine dye</th>
<th>Absorbance change in 10 min with oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,6-Tri-OH BA</td>
<td>&lt;1</td>
<td>No azide</td>
</tr>
<tr>
<td>2,5-Di-OH BA</td>
<td>8</td>
<td>0.06 azide, 0.006 mmol/L</td>
</tr>
<tr>
<td>2,3-Di-OH BA</td>
<td>16</td>
<td>1.16 azide, 0.36 mmol/L</td>
</tr>
<tr>
<td>4-OH BA</td>
<td>40</td>
<td>1.08 azide, 0.24 mmol/L</td>
</tr>
<tr>
<td>2,3,4-Tri-OH BA</td>
<td>44</td>
<td>0.65 azide, 0.36 mmol/L</td>
</tr>
<tr>
<td>3,5-Di-CI-4-OH BA</td>
<td>45</td>
<td>2.14 azide, 0.48 mmol/L</td>
</tr>
<tr>
<td>3-CI-4-OH BA</td>
<td>55</td>
<td>1.75 azide, 0.38 mmol/L</td>
</tr>
<tr>
<td>3,5-Di-CI-2-OH BA</td>
<td>100</td>
<td>3.01 azide, 0.70 mmol/L</td>
</tr>
</tbody>
</table>

*BA = Benzoic acid. a Rate of absorbance changes was linear for 3 min only. b Large reagent blank (0.37).%

References
1. Artiss JD, McEnroe RJ, Zak B. Bilirubin interference in a peroxidase-coupled pro-
2. Maguire GA. Elimination of the "chromo-
gen oxidase" activity of bilirubin oxidase added to obviate bilirubin interference in hy-
drogen peroxide/peroxidase detecting sys-

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Correlation between Calcium and Magnesium in Plasma or Serum?

To the Editor:

Brown (1) and Speich (2) have ques-
tioned the possible correlation between calcium and magnesium, whose physi-
opathological and chemical similarities are so important.

We have studied hypocalcemia and hypomagnesemia in surgical patients, measuring magnesium by the method of Ginder and Heth (3) as modified by Cohen and Daza (4), and calcium by the method of Connerty and Briggs (5) with reagents from Electronucleonics Inc., Fairfield, NJ 07006. The good