Billirubin Inhibition of γ-Glutamyltransferase on the DuPont aca

To the Editor:

The activity of γ-glutamyltransferase (EC 2.3.2.2) is measured on the DuPont aca by an adaptation of the Szasz procedure; γ-glutamyl-p-nitroanilide is transferred to glycylglycine, thereby releasing p-nitroaniline, which is measured at 405 nm. I have noticed low glutamyltransferase activity in patients with cholestasis where alkaline phosphate (EC 3.1.3.1) is increased, total bilirubin > 100 mg/L, and conjugated bilirubin > 75 mg/L. The manufacturer states (1) that icteric serum does not interfere with their methodology for glutamyltransferase.

Combes et al. have stated (2) that conjugated bilirubin and unconjugated bilirubin will depress glutamyltransferase activity significantly. In contradiction of these findings, Dickson and Beck (3), using bilirubin from human bile and crystalline bilirubin, stated that there was no apparent inhibition except at bilirubin concentrations of 140 and 280 mg/L (bilirubin from bile). Their conclusion was that other, unidentified factor(s) contribute to this phenomenon.

Theodorsen and Stromme (4) added bilirubin (source not stated) up to 200 mg/L to serum but found no measurable inhibition of glutamyltransferase activity. They state that the change in absorbance when assaying for glutamyltransferase may be nonlinear in a sample with high background absorbance. Bilirubin alone or in combination with other components of this assay would give a high background absorbance at 400-410 nm. In addition, they report that glutamyltransferase inhibition by donor substrate and acceptor varies with the instrument and methodology.

Analysis of a serum with total bilirubin at 150 mg/L, conjugated bilirubin at 75 mg/L, and glutamyltransferase activity of 44 U/L gave results of 80 and 100 units of glutamyltransferase activity per liter when diluted 10- and 20-fold with an albumin-based diluent. However, assay of several samples with high glutamyltransferase activity diluted with equal volumes of sera having conjugated bilirubin > 75 mg/L and low glutamyltransferase levels showed no inhibition by bilirubin (result always halfway between respective glutamyltransferase levels).

The problem may be due to direct enzymatic interference by conjugated or unconjugated bilirubin (or both) by other unidentified compound(s), to instrumental error from increased background absorbance at 405 nm, or to interference with substrate-enzyme interaction by the inhibitor.

The manufacturer states that icteric serum does not interfere with their methodology; however, in their studies they used crystalline bilirubin, so inhibition by conjugated bilirubin would not be observed.

References

Albert D. Fraser
Regional Laboratory
Dr. Everett Chalmers Hospital
Frederickton, New Brunswick
Canada E3B 5N5

A Simple, Solid-Phase Iodine-125 Radiomimunoassay for Theophylline

To the Editor:

Radioimmunoassay (RIA) offers a convenient, economical method for the assay of theophylline. Cook et al. (1) first described such an assay, in which a tritiated ligand was used. I describe an accurate and simple solid-phase RIA in which the ligand is labeled with 125I.

Insolubilized antibody and 125I-labeled theophylline were obtained from RF Laboratories, Houston, TX 77043, and stored at 4 °C. The antibody was such that 100 μL of the suspension gives 50% binding. The concentration of 125I-labeled theophylline was 400 μCi/L. Buffer was prepared by dissolving 3 g of boric acid, 4.5 g of sodium chloride, 500 mg of disodium ethylene-diaminetetraacetate, and 500 mg of sodium azide in approximately 450 mL of deionized water. After the pH was adjusted to 8.3 with 2 mol/L sodium hydroxide, the buffer was diluted to 500 mL with water. Standards of 0, 1, 5, 10, 20, and 30 mg/L were prepared in theophylline-free serum that had been stabilized with 100 mg of thimerosal per liter and were stored at 4 °C.

After allowing all reagents to reach ambient temperature, 10 μL of each specimen (sample, standard, and control sera) was diluted with 0.5 mL of buffer. One hundred microliters of each diluted specimen was pipetted into 12 × 75 mm borosilicate glass (culture) tubes. To each tube, 100 μL of 125I-labeled theophylline, 100 μL of stirred antibody suspension, and 200 μL of buffer were added. After mixing the contents of the tubes gently, all the tubes were incubated at room temperature for 15 min. After incubation, the tubes were centrifuged for 10 min at 3000 × g, and the supernate was aspirated carefully without disturbing the pellet.

The residue in each tube was counted on a gamma counter until 10 000 cpm had accumulated. A standard curve was constructed on log-log paper and the concentration of the unknowns was determined by comparison with the standard curve.

The sensitivity of the assay is 1 mg/L. The upper limit of the assay is 30 mg/L, and 90% of the counts are displaced. The no-antibody tube (blank) gives 3–6% of the total counts because of the volume of the residual supernatant fluid in the tubes.

The efficiency of the separation of the insolubilized antigen–antibody complex from the free labeled antigen by centrifugation was exhibited by obtaining a variation of less than 3% on triplicate runs. Also, a control serum (Lederle Laboratories, Pearl River, NY 10965) with a nominal concentration of 19.7 mg/L was assayed on seven different occasions over a four-week period. The mean observed concentration was 19.5 mg/L, with a coefficient of variation of 3.3%.

Eighty-six samples were analyzed by the EMIT system and by this RIA method. The coefficient of correlation was 0.99. The regression equation was

\[ y_{\text{EMIT}} = 0.99x_{\text{RIA}} - 0.11 \ (±1.27) \]

Sixteen samples previously assayed by "high-pressure" liquid chromatography (HPLC) were assayed by this RIA method. The coefficient of correlation was 0.99, and the regression equation was:

\[ y_{\text{HPLC}} = 0.94x_{\text{RIA}} + 0.66 \ (±1.2) \]

Several samples in the HPLC lot were lipemic, but this did not affect the RIA results. No theophylline was detected in 10 random samples not known to contain theophylline.

Cross reactivity of the antibody was essentially the same as that reported previously (1). The caffeine absorbed from 10 cups of coffee imbibed at one time, the concentration of which is estimated to be 6.1 mg/L (2), apparently would increase the determined amount of theophylline by less than 1 mg/L. Therefore, the consumption of large amounts of coffee does not preclude meaningful results.

The concept used in enzyme immunoassays such as this EMIT is quite valid. However, a small contamination of samples with reagents, such as contamination that may occur when the tip of a pipet touches the reagent mixtures and
is reused, renders the rest of the kit unusable. This increases the variability of the assay. In this laboratory, standard curves from such runs were in error by 20–50% for the 5 mg/L standard and by about 25% for the 20 mg/L standard; comparable errors for the RIA were 3–5%. Because of the greater immunity of the RIA to this type of analyst error, I prefer the RIA procedure.

The RIA is quite stable as reflected in the low inter assay CV for the Lederle control serum; the month during which it was assayed covers the lifetime of the reconstituted reagents. Results by RIA compare well with both liquid chromatography and EMIT. No elaborate instrumentation is required except for a gamma scintillation counter. Finally, its simplicity facilitates its incorporation into the laboratory routine.

References

Olgah Durón
Presbyterian Intercommunity Hospital
12401 E. Washington Boulevard
Whittier, CA 90602

Evaluation of a New Commercially Available (Ortho) Digoxin Radioimmunoassay Kit

To the Editor:
We evaluated a newly available digoxin radioimmunoassay (RIA) kit ("Ortho Digoxin RIA (PEG) Kit"; Ortho Diagnostics Inc., Raritan, NJ 08889) to see if it performed satisfactorily in an in vitro evaluation of patients' sera in comparison with an established digoxin RIA kit ("Quantitope 125I-Digoxin RIA Kit"; Kallestad Laboratories Inc., Chaska, MN 55318). In doing so we used 100 specimens, as follows:

10 from apparently normal, healthy individuals with no history of cardiac glycoside treatment.
30 with serum digoxin concentrations <1.0 µg/L.
30 with serum digoxin concentrations of 1.0 to 2.5 µg/L.
30 with serum digoxin concentrations >2.5 µg/L.

Samples were arbitrarily placed in the above categories based on results with the Kallestad kit.

All patients' samples were assayed in duplicate, on the same day, by the same technologist, with both digoxin RIA kits.

Briefly, in the Ortho digoxin RIA an iodinated tracer and an antibody are used, with polyethylene glycol separation of the bound fraction. A 50-µL sample is necessary for each tube. In the Kallestad digoxin RIA, an iodinated tracer is also used, but a precomplexed double-antibody step is included, which eliminates the separate addition of a separation agent. A 100-µL sample is necessary for each tube. Each kit method includes a 30-min incubation.

Table 1 summarizes our results. If a serum digoxin value exceeding 2.0 µg/L is accepted as an index of toxicity, results for only one specimen disagreed between kits, and this disagreement was modest (2.2 µg/L with the Ortho kit, 2.0 µg/L with the Kallestad kit).

In this short study, run-to-run variation was as follows (µg/L):

<table>
<thead>
<tr>
<th>Kit</th>
<th>Therapeutic conc.</th>
<th>n</th>
<th>X</th>
<th>SD</th>
<th>CV,%</th>
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</thead>
<tbody>
<tr>
<td>Ortho</td>
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<td>10</td>
<td>0.97</td>
<td>0.03</td>
<td>2.9</td>
</tr>
<tr>
<td>Kallestad</td>
<td></td>
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<td>0.76</td>
<td>0.07</td>
<td>9.2</td>
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<tr>
<td>Toxic conc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho</td>
<td></td>
<td>10</td>
<td>3.03</td>
<td>0.16</td>
<td>5.4</td>
</tr>
<tr>
<td>Kallestad</td>
<td></td>
<td>9</td>
<td>2.62</td>
<td>0.20</td>
<td>7.6</td>
</tr>
</tbody>
</table>

We conclude that there are negligible differences between results obtained with these two kits.

Norman P. Kubasik
Karen Warren
H. E. Sine

The Clinical Laboratories
The Genesee Hospital
224 Alexander St.
Rochester, NY 14607

Falsely Positive Results for Bilirubin with Ames' "Clini-Tek"

To the Editor:
In our laboratory, we recently began using the Ames Clin-Tek 1 reader for routine urinalysis. We obtained several positive bilirubin results on normal-colored urines with the Clin-Tek. These results were rechecked by the IctoTest 1 method and found to be negative for bilirubin. We also obtained positive bilirubin results on some highly colored urines with the Clin-Tek. By the IctoTest method, these were also found to be negative for bilirubin. Since the IctoTest method is more sensitive for bilirubin than the N-Multistix, 1 we are getting false-positive bilirubin results on the Clin-Tek. These false positives occur in about one urine sample out of 40 or 50 samples, or about 2%.

According to a recent evaluation of the Ames Clin-Tek (1), it gave 0% false-positive bilirubins. Since we have found a 2% incidence of false-positive bilirubins with the Clin-Tek, we now check all positive bilirubin results with the IctoTest method. Our instrument has been returned to the company and this has been verified not to be an electronic problem. For this reason we recommend that all users of the Clin-Tek verify all positive bilirubins by an alternative method.

Reference

Alice McDaniel
W. William Spencer

Clinical Laboratories
St. Elizabeth Medical Center
Dayton, OH 45408

An author of ref. 1 above (R.G.) says that they have not encountered this problem. No hyperchromic urines were included in their study. The Ames Co.

1 "Clini-Tek," "IctoTest," and "N-Multistix" are registered trademarks of Ames Co., Division of Miles Laboratories, Inc., Elkhart, IN 46514.