Evaluation of a Solid-Phase Enzyme Immunoassay for Human Choriogonadotropin β Subunit

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We evaluated a quantitative solid-phase enzyme immunoassay for human choriogonadotropin β subunit (β-HCG) with anti-β-HCG:horseradish peroxidase conjugate, recently marketed by Abbott Laboratories. We compared results on 56 patients’ serum specimens, obtained mostly for followup of neoplastic disease, with those by a competitive radioimmunoassay kit. The correlation was good, the differences being of little clinical significance. Linear regression in the low and intermediate ranges gave a slope of 0.93, a y-intercept of 0.34, and a correlation coefficient of 0.97. Precision studies yielded an interassay CV of 6.4% in the intermediate range and 13% in the low range. Sensitivity was 0.69 int. units/L. Cross reactivity was 1 to 2% with specimens fortified with lutropin or follitropin. The only substantial problem was with linearity in the upper part of the standard curve, especially in the interval, 100–200 int. units/L. This problem is obviated by adequate sample dilution.

Human choriogonadotropin (β-HCG) is a sialoglycoprotein hormone, normally secreted by the placenta but also produced ectopically by neoplasms (1). Immunoassays that are more sensitive than the usual pregnancy tests have been used to screen for ectopic pregnancies (2, 3), and quantitative assays have been used for diagnosis and followup of gestational trophoblastic disease (4) and germ cell neoplasms, including choriocarcinoma and testicular cancer (5). However, increased β-HCG concentrations have also been described in serum of patients with cancer of the ovary, cervix (6), prostate (7), stomach, liver, pancreas, breast (1), and other sites. In currently used assays the antibody is directed to the β-subunit, as described by Vaitukaitis et al. (4), but some cross reactivity is seen, especially with lutropin, in many commercial assays (8).

Radioimmunoassays have the disadvantages of waste disposal, limited shelf-life, and need for special equipment. Consequently, enzyme immunoassays have been developed in which either the antigen or antibody is labeled. Assays have been described in which β-HCG:enzyme conjugates are used, the sensitivity of which is comparable with that of conventional RIA (9, 10). VanWeemen and Schuurs (11) developed a β-HCG assay with antibody–enzyme conjugate, but its sensitivity was less than that of enzyme immunoassay involving β-HCG:enzyme conjugate. Wada et al. (12) recently described an assay involving solid-phase antibody for the α-subunit and enzyme-coupled monoclonal antibody for the β-subunit. Its sensitivity was good, but cross reactivity with lutropin was greater than with the same antibody used in competitive RIA. Abbott Laboratories recently developed a solid-phase enzyme immunoassay with anti-β-HCG:horseradish peroxidase conjugate. We compared this assay with a competitive RIA, with the results reported here.

Materials and Methods

Enzyme immunoassay: With the kits we used (Abbott Laboratories Diagnostics Division, North Chicago, IL 60064), the procedure is briefly as follows. The standards, controls, and patients’ samples are first incubated for 90 min with anti-β-HCG immobilized on polystyrene beads, then washed and incubated for 60 min with anti-β-HCG:horseradish peroxidase conjugate, and washed again to remove the excess. An o-phenylenediamine substrate and hydrogen peroxide are added and the resulting color is measured spectrophotometrically at 492 nm. The quantitative procedure in the package insert was used with duplicate specimens; standards are provided. The “Quantum II” spectrophotometer and automatic data processor used in this procedure were from Abbott. Their kit insert lists a sensitivity of ≤1.1 int. units/L and a linearity to 200 int. units/L.

Comparison radioimmunoassay: The method we used for comparison was the βIII-HCG” kit (Serono Laboratories Inc., Randolph, MA 02368), a quantitative RIA in which a precipitating solution contains a second antibody and polyethylene glycol. Standards and positive and negative controls are provided in the kit. We followed the procedure recommended by Serono for “Method II” for the kit (overnight incubation). Its sensitivity in our laboratory is ≤1.56 int. units/L. In both methods the standards are calibrated against the World Health Organization Second International Standard, in which 5 int. units/L is equivalent to 1 μg/L.

Patient comparison: We analyzed, by both methods, 43 serum specimens from 40 men whose cases were being followed for testicular cancer and 13 specimens from nine women (five with gestational trophoblastic disease or choriocarcinoma, three with threatened abortions, one with abdominal pain). This population included 23 specimens with values <1.56 int. units/L, 14 specimens with values of 1.56–30.0 int. units/L, and 19 specimens with values >30.0 int. units/L by RIA.

Sensitivity: We assessed the sensitivity of the enzyme immunoassay by 20 within-assay replications of the “zero” calibrator. The mean result plus 2 SD was considered the lower limit of detection (sensitivity).

Precision: We used data from 24 interassay and eight intra-assay replications of Immunoassay Control Serum Level I (low) and 16 interassay and 12 intra-assay replications of Level II (intermediate) to calculate CV.

Linearity: Three patients’ specimens and a College of American Pathologists’ survey specimen were serially diluted with serum diluent (“zero” calibrator) and assayed, to determine linearity.

Recovery: Known β-HCG standards from the Serono kit,
ranging from 5 to 200 int. units/L, were diluted twofold with either Abbott's serum diluent or a patient's serum with ≤1 int. unit/L of β-HCG.

**Cross reactivity:** Lutropin, follitropin, and thyrotropin standards were diluted with serum diluent or a patient's serum containing ≤1 int. unit/L of β-HCG per liter, then assayed for β-HCG. To calculate percent cross reactivity, 1 int. unit/L (sensitivity) was subtracted and this result was divided by the final concentration of lutropin, follitropin, or thyrotropin.

**Results**

Comparison of 23 specimens with β-HCG results <1.56 int. units/L (by RIA) to the enzyme immunoassay method showed good correlation. For 20 of these specimens, the enzyme immunoassay also gave results <1.56 int. units/L, while in the other three, results were also low (2.0–2.2 int. units/L). One of these three patients had evidence of disease by radiologic tests, and another did have residual embryonal carcinoma and teratoma at surgery.

Comparison of 14 specimens with intermediate concentrations of β-HCG also showed good correlation. Three with RIA results of 1.56–3 int. units/L had enzyme immunoassay results of ≤1 int. unit/L: two had no evidence of clinical disease, while the third had an alpha-fetoprotein of 849 μg/L (possible false negative). With RIA as the x-axis, linear regression of results for the other 11 specimens gave a slope of 0.93, a y-intercept of 0.34, and a correlation coefficient of 0.97. The paired-t value was −0.475, not significant (NS). The 19 specimens with high values showed greater disparity, especially in the range >1000 int. units/L, but these differences were not clinically significant. For example, the highest values were found in a pregnant female with threatened abortion; the RIA result was 46 800 and the enzyme immunoassay was 89 700 int. units/L (with 1000-fold dilutions). For results in the 100–200 int. units/L range, enzyme immunoassay results were closer to RIA results when specimens were first diluted (see linearity results). For the range from 30 to 30 000 int. units/L, the paired-t value was 0.996 (NS). Combining the intermediate and high groups gave a paired-t value of 0.996 (NS). Linear regression was not performed on the entire group because of the extremely wide range and the necessity of performing dilutions in the high range.

Sensitivity as determined by replicates of the “zero” standard was 0.69 int. unit/L. Precision studies for the Level I control yielded a mean of 2.2 int. units/L, an interassay CV of 13% (SD 0.32), and an intra-assay CV of 6% (SD 0.13). With the Level II control, the mean was 18.3 int. units/L, with an interassay CV of 6.4% (SD 1.2) and an intra-assay CV of 6.5% (SD 1.2). Previous interassay precision studies with the RIA method gave a CV of 41% in the Level I control and a 9.5% CV at a higher range.

Good linearity of results vs concentration was found in all patients’ samples and the survey specimen up to at least 50 int. units/L. With one patient and the survey specimen, linearity was maintained from 64-fold dilution to the undiluted values of 151 and 154 int. units/L, respectively. Linearity was lost in the other two, with twofold diluted and undiluted specimens giving lower calculated results especially. One specimen gave calculated results of 73, 112, 172, and 226 int. units/L undiluted and at two-, four-, and eightfold dilutions, respectively. The other gave results of 140, 173, 255, and 262 int. units/L undiluted and at two-, four-, and eightfold dilutions. The undiluted assay did not register >200 int. units/L, as one would expect from the results at higher dilutions and the RIA result. Lack of linearity of response to undiluted specimens and 10-fold dilutions was also noted for two other specimens in the range of 100–200 int. units/L by RIA.

Recovery studies yielded an average recovery of 94% with a range of 74–111%. Lowest recoveries (74 and 80%) were found with the lowest (4 int. units/L) and highest (200 int. units/L) Serono standards. Cross-reactivity studies showed no significant cross reactivity with thyrotropin concentrations of 2.5 and 25 int. units/L. At final lutropin concentrations of 30 and 150 int. units/L, cross reactivities were 1.0 and 1.5%, respectively; with follitropin at 30 and 165 int. units/L, cross reactivity was 1.7%.

**Discussion**

Abbott’s enzyme immunoassay method for β-HCG is technically easier than many RIA methods, because its fewer steps involve less critical pipetting. Also only 3 h of incubation is required, compared with the overnight incubation for the Serono method. Finally, the longer shelf-life of enzyme immunoassay reagents and the elimination of radioactive waste disposal and gamma counters are advantageous. Overall cost per test in our laboratory would be less than for an RIA.

The enzyme immunoassay gave good results overall. Our calculated sensitivity (≤0.69 int. unit/L) was less than the 1 int. unit/L listed in the kit insert and for the RIA method. Precision was better than with the RIA method, especially in the low range (CV 13% vs 40%). Patient comparison studies showed few significant clinical differences. When slight differences were found in the critical lower range (<5 int. units/L), the enzyme immunoassay result more often correlated with the clinical impression. In the upper range, the discrepancies were not clinically important, and the large dilutions required (up to 1000-fold) tend to magnify any differences between the methods. Recovery studies had acceptable results, and cross-reactivity studies showed low (1 to 2%) cross reactivity with lutropin and follitropin. This is similar to cross-reactivity results for the RIA, and is better than in other antibody-linked enzyme immunoassay reports (12).

The only substantial problem encountered was with linearity in the upper part of the standard curve, especially 100–200 int. units/L, which was obviated by adequate dilution with serum diluent. This was not a problem with all patients’ samples and did not occur with survey specimens or standards. Nonspecific binding to the bead might cause some of the antibody sites to be unavailable for reaction with β-HCG and conjugate. Altered forms and fragments of β-HCG have been described in patients with neoplasms (13) and might theoretically cause problems with this type of assay, or other serum factors could be responsible. To correct for this problem, we would suggest diluting samples in the high range of the standard curve. The upper limit before diluting may vary among patient populations and should be determined by the individual laboratories. We would recommend dilutions, at least for values >100 int. units/L.

**References**

4. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay for


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**Quality-Control Sera for Routine Determination of Aluminum by Electrothermal Atomic Absorption Spectroscopy**

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Several commercially available quality-control sera were analyzed for aluminum content by atomic absorption spectroscopy with a stabilized-temperature graphite furnace. The values obtained ranged between 4 and 1250 μg/L (0.148 to 46.235 μmol/L). No significant difference was detected for between-vial variation for four lots of quality-control sera (p > 0.05). Control sera stored in 1-mL polypropylene vials and frozen at −20 °C for up to six months showed no significant variation in aluminum content (p > 0.05), but those stored in their original glass containers had significantly increased aluminum content (p < 0.001) over a four-week period.

The accumulation of aluminum in patients with chronic renal failure reportedly contributes to dialysis encephalopathy and to osteomalacia that is resistant to vitamin D therapy (1–3). The therapeutic removal of aluminum with desferrioxamine and dialysis improves these patients' well-being significantly (4, 5). The method of choice for monitoring concentrations of aluminum in serum for the diagnosis and treatment of these patients is atomic absorption spectrometry with use of a graphite furnace (6, 7).

The lack of reference material and an assayed quality-control sera to ensure the reliable determination of serum aluminum by atomic absorption spectrometry has prompted us to develop and to search for suitable sera to fill this need. We selected without conscious bias 13 commercially sup-

Cited references to the study:

1. Donaldson et al. (1980).

2. Weintraub et al. (1980).

3. Leung et al. (1982).

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