Simple Quantitative Measurement of Serum Choriogonadotropin Compared with Immunoradiometric, Immunoenzymometric, and Chemiluminescent Assays

R. J. Norman, T. A. Gilmore, and J. W. McLoughlin

We evaluated a new simplified quantitative method (Tandem Icon QSR; Hybritech) for choriogonadotropin (hCG), which could theoretically be performed in a ward or with outpatients. The method was compared with immunoradiometric (Biocline Australia), chemiluminescent (Amerlite; Amersham), and immunoenzymometric assays (Status; Dade). We analyzed by each of the methods 104 serum samples from pregnant and nonpregnant patients. For quantitative hCG values indicative of pregnancy (>25 int. units/L), excellent correlation was observed between the Tandem Icon method and the other standard laboratory assays \( (r = 0.995, 0.990, \) and \( 0.992, \) respectively). Occasional problems arose because of the instability of Tandem Icon QSR reagents at room temperature but this was resolved by storing the reagents at \( 4^\circ \)C. We conclude that this simplified quantitative method for hCG is reliable and suitable for use outside of the routine immunoassay laboratory.

Measurement of human choriogonadotropin (chorionic gonadotropin, hCG) is an important diagnostic component in obstetrics and gynecology \( (1, 2) \).\(^1\) Qualitative assays are used in the diagnosis of pregnancy, whereas quantitative results are valuable in the assessment of bleeding in early pregnancy, the differential diagnosis of ectopic pregnancy, and the management of gynecological malignancies \( (3, 4) \). Qualitative assessment of hCG has been revolutionized by the application of monoclonal antibodies and immunoenzymometric methods. Relatively small quantities of hCG now can be determined in the presence of high concentrations of other gonadotropins such as lutropin (luteinizing hormone) and follitropin (follicle-stimulating hormone) and without the necessity for a specialized laboratory with staff skilled in radioimmunoassays \( (5-8) \). As a result, qualitative pregnancy tests are reliable, sensitive, and specific and can be conveniently used for tests in outpatients \( (9) \). Quantitative measurements of hCG in outpatients have been more difficult because of the necessity for a standard curve and quality controls in such estimates.

The immunoconcentration method of assay, in which one antibody is incorporated onto a membrane and the other is labeled with an enzyme to produce a color when substrate is added, offers the potential for reliable and accurate quantitative measurement of hCG \( (10, 11) \). We decided to evaluate this method in a clinical setting and to compare the results with those of laboratory methods, including immunoradiometric (IRMAs), immunoenzymometric (IEMAs), and chemiluminescent assays (CLAs). Our criteria for a clinically useful assay were that it should be simple to perform, it should correlate well with accepted laboratory methods, and it should correctly classify subjects as to pregnancy status.

Materials and Methods

Samples

Serum was obtained from 82 subjects subsequently shown to have a normal intra-uterine pregnancy and from 22 subjects with equivocal or negative quantitative hCG results, who were subsequently shown not to be pregnant. All were assayed with the Tandem Icon QSR and the laboratory methods described below. Samples with high concentrations of hCG were diluted as recommended by the manufacturers. The range of hCG values in test serum samples is shown in Table 1.

All subjects who were pregnant exhibited Tandem Icon hCG values >25 int. units/L, whereas those who were subsequently shown not to be pregnant had values <25 int. units/L. These results were then compared with those from the laboratory assays. All hCG standards were calibrated against the First International Reference Preparation (IRP 75-537).

Tandem Icon Quantitative Method

The Tandem Icon QSR kit and Icon reader were obtained from Hybritech, West Ryde, N.S.W., Australia. This solid-phase immunoenzymometric assay, which measures intact hCG, uses two monoclonal antibodies and a calibrated test cartridge. Serum sample and pretreatment solution are added to the Icon membrane on which the first monoclonal antibody is immobilized. After filtration, the second monoclonal antibody (which is linked to alkaline phosphatase) is added. After a short

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<table>
<thead>
<tr>
<th>Table 1. Range of hCG Values and Numbers of Samples Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range, int. units/L</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>0–25</td>
</tr>
<tr>
<td>25–10^2</td>
</tr>
<tr>
<td>10^3–10^3</td>
</tr>
<tr>
<td>10^4–10^4</td>
</tr>
<tr>
<td>10^5–10^6</td>
</tr>
<tr>
<td>&gt;10^6</td>
</tr>
</tbody>
</table>

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\(^1\) Nonstandard abbreviations: hCG, human choriogonadotropin; IRMA, immunoradiometric assay; IEMA, immunoenzymometric assay, and CLA, chemiluminescent assay.

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incubation (2 min) and a wash step to remove unbound enzyme-linked antibody, substrate is added. The reflectance of the resulting color developed may then be read quantitatively in the Icon reader after 3 min. Quantitative results are obtained by initially calibrating the reader with an internal reference spot on the cartridge (>200 int. units/L). Subsequent readings compare the reference and test spots by reflectance densitometry. The range of the assay extends from 5 to 300 int. units of hCG per liter.

Comparison Laboratory Methods

**Immunoradiometric assay.** The IRMA kit, obtained from Biocline Australia, Marrickville, N.S.W., is a solid-phase assay that measures both intact hCG and the free beta subunit. We used a Multi Gamma II 1260 gamma counter (LKB Wallac, Turku, Finland) to measure 125I radioactivity. The internal software of the gamma counter calculated values by fitting a curve for counts per minute vs concentration.

Samples, standards, and controls are incubated for 60 min at 37 °C with two monoclonal antibodies, one labeled with 125I and the other covalently linked to magnetizable particles. After incubation, the unbound labeled antibody is removed by decanting after magnetic sedimentation or by centrifugation. The radioactivity in the washed precipitate is counted and the hCG concentration is calculated from the standard curve. The range of the assay extends from 5 to 500 int. units/L. Intra-assay coefficients of variation (CVs) were 4% at 26 int. units/L and 2% at 229 int. units/L. Interassay CVs were 11% at 15 int. units/L and 6% at 221 int. units/L. The least detectable quantity of hCG in this assay is 1 int. unit/L.

**Chemiluminescent assay.** The Amerlite kit and analyzer were obtained from Amer sham Australia, Sydney, N.S.W. This solid-phase chemiluminescent assay uses polyclonal and monoclonal antibodies to measure intact hCG and the free beta subunit.

Serum samples and standards are pipetted into microtiter wells coated with polyclonal antibody. Monoclonal antibody linked to horseradish peroxidase is added to the wells, and samples are incubated on a shaker at 37 °C for 15 min. (A 60-min incubation procedure may be used.) The wells are aspirated and washed automatically by the plate washer to remove unbound enzyme-linked antibody. Signal reagent containing a luminescent substrate and an enhancer is added. Plates are incubated for 2 to 20 min before the luminescence produced is read in the Amerlite analyzer. The amount of light produced is measured and hCG concentration is calculated from a standard curve that has been assayed previously and stored. (Six standards must be assayed in duplicate initially and this curve is stored in the analyzer. Subsequent assays need contain only four standards in singleton, which are compared with the original curve; provided certain criteria are met, these results are used to create a new curve by extrapolation, which is used to calculate the hCG concentration.) The range of the assay extends from 5 to 1000 int. units/L.

Intra-assay CVs were 3% at 78 int. units/L and 5% at 144 int. units/L; interassay CVs were 26% at 10 int. units/L and 13% at 234 int. units/L.

**Immunoenzymometric assay.** The Stratus kit and Stratus automated analyzer were obtained from Pacific Diagnostics, Brisbane, Qld., Australia. This fully automated fluorometric assay measures intact hCG.

The serum sample is transferred to a sample carousel where it is loaded automatically onto the Stratus analyzer. The sample is transferred to a tab containing monoclonal antibody linked to glass fiber paper, and then a second monoclonal antibody linked to alkaline phosphatase is added. After a short incubation of 3 min within the analyzer, a wash solution containing substrate is added to remove unbound enzyme-linked antibody. The tab is then conveyed to the optical unit where its fluorescence is read by front-surface fluorometry. The hCG concentration is calculated from a standard curve initially assayed and stored in the analyzer. These curves are stable for several weeks. The range of the assay extends from 5 to 500 int. units/L. Intra- and interassay CVs were 2% at 27 int. units/L, 2% at 38 int. units/L, and 14% at 185 int. units/L, respectively.

**Statistical Analysis**

Results were logarithmically transformed to compensate for the spread in data values. Pearson's correlation and regression analysis were then performed on all data sets by using Statgraphics (STSC PLUS*WARE statistical graphics system; Statistical Graphics Corp, STSC, Inc., 1986) software package. Sensitivity, specificity, and predictive indices were calculated for each test (13). Sensitivity was defined as the percentage of pregnant subjects with a positive hCG result (>25 int. units/L), specificity as the percentage of nonpregnant subjects with a negative hCG result (<25 int. units/L), and positive predictive value as the percentage of pregnant subjects with a positive hCG value out of all subjects with positive test results.

**Results**

The simplified quantitative method (Tandem Icon QSR) was easy to use and gave results within 10 min of sample addition. However, we encountered some initial methodological problems. Because it was not possible to use plasma from EDTA anticoagulant tubes, we used serum or heparinized plasma. Also, one fifth of the samples initially had to be reassayed because of instability in the internal reference standard of the Icon cartridge. This was remedied by storing all reagents at 4 °C rather than at room temperature as advocated by the manufacturers. Subsequent batches were consistently reliable under these storage conditions.

**Accuracy and precision.** Aliquots of two samples assayed repeatedly (n = 14) within the same run on the same day with Icon cartridges gave CVs of 22% at 14 int. units/L and 15% at 144 int. units/L. The CV of the reflectance reader alone was <2%.

Dilution of a sample with a high concentration of hCG showed parallelism with the standard curve. Addition of
known amounts of hCG (250 int. units/L, n = 4) to serum samples gave analytical recoveries of about 100% (76–104%).

Correlation with the laboratory methods. Results were subdivided into those >25 int. units/L ("positive pregnancy test") and those <25 int. units/L ("equivocal or negative"), a commonly used laboratory criterion for whether the laboratory estimate of significant hCG secretion is positive or negative (13).

Results with the Icon correlated well with those of other methods for results >25 int. units/L, but showed greater variability in samples with lesser concentrations (Table 2). One sample with results of 26 int. units/L by IRMA, 22 by CLA, and 26 by IEMA was 14 int. units/L by the Icon. Another was 23 int. units/L by IRMA, 19 by CLA, and 31 by IEMA, but 35 int. units/L by the Icon.

The IRMA classified all specimens correctly, whereas the CLA, the results of which correlated well with those of the IRMA, misclassified three pregnancy samples as negative; the IEMA misclassified two pregnancy samples as negative. The positive predictive value of the Icon overall was 98.7%.

Table 2 indicates that $r^2$ and the slope of the regression line were poor when serum values of hCG were <25 int. units/L. Most of the results between 5 and 25 int. units/L for the Icon were questionable, whereas results in this range from the quantitative laboratory assays were more reliable. Table 3 shows the sensitivity, specificity, and predictive indices of the tests compared with the IRMA.

Discussion

Quantitative assessment of hCG concentrations is frequently required when faced with a clinical problem such as the differential diagnosis of ectopic pregnancy, bleeding in early pregnancy, or detection of gynecological tumors. Small laboratories and rural centers are not able to offer daily quantitative assays of hCG and even in large institutions, such assays are not usually cost effective when offered. This study investigated alternatives to formal traditional quantitative procedures.

Table 2. Correlation of Icon Quantitative Method (y) with Other Quantitative Laboratory Methods (x)

<table>
<thead>
<tr>
<th>x</th>
<th>n</th>
<th>r</th>
<th>$r^2$</th>
<th>Slope</th>
<th>y-Intercept</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>All results</td>
<td>100</td>
<td>0.986</td>
<td>97.3</td>
<td>1.04</td>
<td>-0.2</td>
<td>0.137</td>
</tr>
<tr>
<td>IRMA</td>
<td>103</td>
<td>0.992</td>
<td>98.2</td>
<td>0.98</td>
<td>-0.1</td>
<td>0.135</td>
</tr>
<tr>
<td>CLA</td>
<td>81</td>
<td>0.999</td>
<td>98.3</td>
<td>0.95</td>
<td>0.0</td>
<td>0.120</td>
</tr>
<tr>
<td>IEMA</td>
<td>81</td>
<td>0.992</td>
<td>98.3</td>
<td>0.95</td>
<td>0.0</td>
<td>0.120</td>
</tr>
<tr>
<td>Results &gt;25 int. units/L</td>
<td>100</td>
<td>0.995</td>
<td>99.0</td>
<td>0.99</td>
<td>0.0</td>
<td>0.120</td>
</tr>
<tr>
<td>IRMA</td>
<td>78</td>
<td>0.990</td>
<td>97.9</td>
<td>0.93</td>
<td>0.28</td>
<td>0.155</td>
</tr>
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<tr>
<td>Results &lt;25 int. units/L</td>
<td>100</td>
<td>0.995</td>
<td>99.0</td>
<td>0.99</td>
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Few studies have assessed the value of simple methods of measuring hCG quantitatively with immunoncentration cartridge technology, despite their commercial availability. These tests are rapid (<10 min) and involve a simple, inexpensive reflectance densitometry meter. The Tandem Icon method could easily be carried out by laboratory and clinical staff inexperienced in immunoassay technology, indicating the possibility of performing the test in a ward or simple laboratory. The Icon correlated well with the IRMA, particularly when results were >25 int. units/L. The slope of 0.99 and intercept of 0 indicate a close predictive agreement. Both the controls and 0.5 dilutions of each test were run to establish a baseline for the range less reliable. However, other quantitative methods such as the chemiluminescent and immunoenzymometric assays encountered equal difficulty, indicating that hCG measurement in this range is problematic. Despite these observations, the sensitivity and specificity of the Icon test was quite acceptable for clinical classification of pregnancy status, and for values >25 int. units/L (when quantification is required); agreement with conventional methods was excellent. The precision of the Icon was not as good as would be expected for comparable quantitative immunoassays such as the IRMA. However, sequential monitoring of subjects with suspected ectopic pregnancy or threatened abortion makes use of the doubling time of hCG of ~48 h in normal pregnancy; this method would be quite adequate for these purposes.

The cost of equipment was high for both the CLA and IEMA and, although both kits generally performed well overall, they were not suited for use outside of a specialized immunoassay laboratory.
Provided pregnancy samples alone are measured with the Tandem Icon QSR, no difficulties result from the measurement of intact hCG rather than the inclusion of free beta subunit. If tumors are measured, it is possible that those patients who have predominant amounts of free beta subunit in the circulation will have misleading results by the Icon QSR. We noted little difference between the methods reported in this study, some of which are intact-hCG specific and others that detect the beta epitope in intact and free subunits.

These methods were performed in an immunoassay laboratory, and although the results of the Icon were promising, the feasibility of their use in a ward or clinic should be established. Some potential problems are the necessity for centrifugation of the sample and the accurate pipetting of serum onto the cartridge. However, in a preliminary study, we gave the Icon test kit to a clinician with no previous laboratory experience and only 5 min of instruction on the Icon method. We compared 20 results with those obtained by trained technologists. All results were within 10% of the laboratory results, confirming the potential for use outside of a specialized laboratory.

In summary, at least one of the commercially available cartridges for hCG measurement offers the potential for rapid and simple quantitative assay of hCG. Although results from the Tandem Icon QSR at the extreme lower end showed a degree of imprecision similar to that of other nonradioisotopic kits, this method is well suited for use by laboratories or clinics with a requirement for accurate and reliable measurement of serum or plasma hCG because its results correlate well with those of the comparative method, it gives rapid results, and because it is easy to perform. Urine hCG immunoconcentration cartridges remain the test of choice for qualitative assessment of pregnancy status (13), but use of the Tandem Icon QSR appears to offer simple and reliable quantitative measurement of plasma concentrations of hCG. "Bedside" biochemistry, widely used by clinical staff for routine urinalysis and blood glucose monitoring, may also find application to hormone measurement in wards or smaller laboratories not usually offering hormone tests.

We thank the various kit manufacturers for donating some of the reagents.

References