An Evaluation of Four Serum Tests for Pregnancy

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We evaluated four pregnancy tests (Biocept-G, Beta-CG, Preg/Stat, and HCG-Beta Screen), using sera from 59 non-pregnant subjects and 77 patients with serum human chorionic gonadotropin beta-subunit (β-hCG) concentrations ranging from 4 to 100,000 int. units/L. The results obtained for each test were compared with the results predicted on the basis of the sample’s β-hCG concentration and the β-hCG concentration the manufacturer claimed necessary for a positive result (the test’s analytical sensitivity). Biocept-G had the best sensitivity (100%), specificity (99.9%), and accuracy (99.2%). Beta-CG had the poorest sensitivity (86.4%), Preg/Stat the poorest specificity (87.5%), and accuracy (92.6%). We confirmed the manufacturer’s claimed analytical sensitivity (200 int. units/L) for the Biocept-G procedure, but our calculated analytical sensitivity for the other tests was significantly different from that claimed by their manufacturers. Best results were obtained with Biocept-G, but with its analytical sensitivity of 200 int. units/L, samples from early pregnancy will give negative results. None of the pregnancy tests evaluated here will establish the presence or absence of early pregnancy with certainty.

Additional Keyphrase: chorionic gonadotropin

In the past decade numerous tests have been developed to detect early pregnancy. In contrast to other types of pregnancy tests, early pregnancy tests are performed on serum specimens and involve either human chorionic gonadotropin (hCG) radioreceptor assays or hCG beta-subunit (β-hCG) radioimmunoassays. These tests are reputed to give positive results for pregnancies of two weeks’ duration or less. Although most studies so far have found that the use of early pregnancy tests generally improves the management of the complications of early pregnancies (for example, ectopic pregnancies), these reports vary considerably in their evaluations of the usefulness of such tests (1–13). Possible sources of the variability include (a) differences between methodologies, (b) differences in the serum β-hCG concentration required to give a positive test result (the analytical sensitivity of the test), (c) differences in patient populations, or (d) some combination of these.

In this study we have calculated sensitivity, specificity, and accuracy for four serum pregnancy tests. One of these tests was a radioreceptor assay and the other three were radioimmunoassay procedures. The analytical sensitivity assigned to these tests by the manufacturer varied from 10 to 200 milli-int. units of β-hCG per milliliter of serum (int. units/L). Our study population consisted of patients carrying pregnancies at a wide range of gestational ages, as well as nonpregnant control subjects. We also examined the accuracy of the analytical sensitivity claimed by the manufacturer for each of the four tests evaluated in this study.

Materials and Methods

Definitions: “Analytical sensitivity” refers to the minimum concentration of β-hCG that will give a positive test result. The analytical sensitivity reported by the manufacturer of the test is the “claimed analytical sensitivity”; the analytical sensitivity we calculated from experimental results is the “calculated analytical sensitivity” of the test.

A “true positive” or “false negative” is a positive or negative pregnancy test result, respectively, when the patient’s β-hCG concentration (as measured by the reference method) is greater than the claimed analytical sensitivity for that test. A “false-positive” or “true-negative” result is a positive or negative pregnancy test result, respectively, when the patient’s β-hCG concentration (as measured by the comparison method) is less than the claimed analytical sensitivity for that test.

The “sensitivity” of the pregnancy test is the number of true-positive results divided by the sum of the true-positive plus false-negative results. The “specificity” of the pregnancy test is the number of true-negative results divided by the sum of the true-negative and false-positive results. “Accuracy” is calculated by dividing the number of true-positive plus true-negative results by the total number of samples tested (true-positive plus true-negative plus false-positive plus false-negative results).

Comparison procedure: The comparison method used in this study was a quantitative measurement of the serum β-hCG concentration, with reagents manufactured by Serono Laboratories, Inc., Braintree, MA 02184. All serum specimens were analyzed in duplicate and the results averaged to obtain the final result. Tests were repeated when the duplicate answers did not agree within 5%. With this procedure the minimum detectable concentration is 3 milli-int. units of β-hCG per milliliter of serum.

Pregnancy tests: We used the following serum pregnancy tests in this study:

1. HCG-Beta Screen; Nuclear Medical Systems, Inc., Newport Beach, CA 92663. This is a β-hCG radioimmunoassay procedure with a claimed analytical sensitivity for β-hCG of 10 int. units/L of serum.
2. Preg/Stat HCG-Beta RIA Kit; Serono Laboratories, Inc. This is a β-hCG radioimmunoassay procedure with a claimed analytical sensitivity for β-hCG of 25 int. units/L of serum.
3. Beta-CG Pregnancy Assay; Monitor Science Corp., Newport Beach, CA 92663. This is a β-hCG radioimmunoassay procedure with a claimed analytical sensitivity for β-hCG of 40 int. units/L of serum.
4. Biocept-G; Wampole Laboratories, Cranberry, NJ 08512. This is an hCG radioreceptor assay with a claimed analytical sensitivity for hCG of 200 int. units/L of serum.

All analyses were performed by experienced clinical laboratory personnel with strict attention to manufacturer’s directions. Each of the pregnancy tests used in this study furnished both a positive and negative control. When the counts per minute (cpm) in the patient’s sample tube was

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less than the cpm for the positive control, the result was reported as positive; when the cpm for the patient's tube exceeded the positive control, a negative result was reported. Each test also had a small "indeterminate" range.

Patients and control subjects: The 29 patients in this study were all being treated at a pregnancy termination clinic. To obtain sera with a wide range of β-hCG concentrations, blood samples were obtained just prior to pregnancy termination and at weekly intervals for up to four weeks afterwards. A total of 78 blood samples were obtained from these patients. The control group was 59 (57 women, two men) nursing, secretarial, and laboratory all known not to be pregnant. In all cases serum was promptly removed from the clot and frozen at −20 °C until analyses were performed.

Results

The distribution of the β-hCG concentrations for the 136 serum samples used in this study is shown in Figure 1. Fifty-nine (43%) of the sera were from nonpregnant subjects and had no detectable β-hCG. In 33 (24%) of the samples β-hCG concentrations were between 3 and 200 int. units/L, 12 (9%) were between 200 and 1000 int. units/L, and 32 (24%) were greater than 1000 int. units/L. The results of pregnancy tests performed on these samples are in Table 1. The sensitivity, specificity, and accuracy of each test, based on our test population, are given in Table 2.

In our attempt to verify the claimed analytical sensitivity of each test, we examined the results of each test for different ranges of serum β-hCG concentrations. All results of all tests were positive when the serum β-hCG concentration exceeded 200 int. units/L. The percent of positive results for each test for samples with low β-hCG concentrations (<200 int. units/L) are in Table 3. Our calculations of the sensitivity and accuracy for each test (Table 3) were based on a patient population that we considered typical for purposes of pregnancy testing. However, the calculated sensitivity and accuracy for a test may be different if applied solely to special populations (e.g., in vitro fertilization, normal advanced pregnancies). Using Table 3, one may estimate the test's sensitivity and accuracy for such populations.

We calculated the analytical sensitivity of each pregnancy test on the basis of the β-hCG concentration at which 50% of the results for that test were positive. As seen in Figure 2, there was excellent correlation between the claimed analytical sensitivity and the calculated analytical sensitivity for the Biocept-G procedure. We found that the calculated analytical sensitivity for the Beta-CG procedure was more than double the manufacturer's claimed analytical sensitivity for this procedure. For the other two methods, Preg/Stat and HCG-Beta Screen, the calculated analytical sensitivity was about half of the claimed analytical sensitivity for the test.

Table 2. Diagnostic Sensitivity, Specificity, and Accuracy of the Serum Pregnancy Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocept G</td>
<td>100.0</td>
<td>98.9</td>
<td>99.2</td>
</tr>
<tr>
<td>Beta-CG</td>
<td>86.4</td>
<td>98.6</td>
<td>93.1</td>
</tr>
<tr>
<td>Preg/Stat</td>
<td>98.4</td>
<td>87.5</td>
<td>92.6</td>
</tr>
<tr>
<td>HCG-Beta Screen</td>
<td>98.6</td>
<td>88.9</td>
<td>94.1</td>
</tr>
</tbody>
</table>

Fig. 2. Percentage of patients with a positive pregnancy test result for different ranges of serum β-hCG concentrations

Calculated analytical sensitivity (int. units/L): HCG-Beta Screen (---), 6; Preg/Stat (--), 13; Beta-CG (--), 85; Biocept G (-----), 200
Table 3. Results of Serum Pregnancy Tests for Different Ranges of Serum β-hCG Concentration

<table>
<thead>
<tr>
<th>β-hCG concentration (units/L)</th>
<th>Biocept G (200)*</th>
<th>Beta-CG (40)</th>
<th>Preg/Stat (25)</th>
<th>HCG-Beta Screen (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;200</td>
<td>44 (100)</td>
<td>44 (100)</td>
<td>44 (100)</td>
<td>44 (100)</td>
</tr>
<tr>
<td>100–199</td>
<td>0 (0)</td>
<td>5 (92)</td>
<td>6 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>50–99</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>6 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>25–49</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (88)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>10–24</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (90)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>4–9</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (67)</td>
</tr>
</tbody>
</table>

*a Number of specimens in each group.
*b Numbers in parentheses by each test name are the claimed analytical sensitivity of the test (int. units/L).

Discussion

Although the results of serum pregnancy tests are reported as either “positive” or “negative,” we emphasize that such a report does not necessarily indicate the presence or absence of pregnancy. The determination of what constitutes either a “positive” or “negative” result is affected by both the analytical sensitivity of the procedure as well as the sensitivity and specificity of the test. Serum β-hCG concentration normally being less than 3.0 int. units/L, the ideal early pregnancy test should have a very low analytical sensitivity (<10 int. units/L). All sera with β-hCG concentrations greater than this would give a positive result; less than this would give a negative result. Unfortunately, none of the four tests evaluated here fulfilled these criteria for the ideal pregnancy test.

Although the sensitivity, specificity, and accuracy of the Biocept-G procedure are excellent, this test has a relatively high analytical sensitivity for β-hCG (200 int. units/L of serum). In a setting where the serum pregnancy test is used merely as a diagnostic aid to confirm pregnancy after the time of the first missed menstrual period, the use of this test is appropriate. However, most early pregnancy tests have more general applications and are particularly important as an aid in the diagnosis of ectopic pregnancies. Ruptured ectopic pregnancy represents a medical emergency that is still associated with significant morbidity and mortality (14–15). The serum concentration of β-hCG is well known to be less than the analytical sensitivity for the Biocept-G procedure in a significant percentage of patients with ectopic pregnancies (4, 7, 10). The other three tests (HCG-Beta Screen, Beta-CG and Preg/Stat), although having lower claimed analytical sensitivities (β-hCG at 40 int. units/L of serum or less) than Biocept-G, suffer from a relatively low sensitivity, specificity, and (or) accuracy. HCG-Beta Screen and Preg/Stat had the poorest specificities, Beta-CG had the poorest sensitivity, and Preg/Stat had the poorest accuracy of the four procedures evaluated here. We therefore consider it inapposite to report the results of one of these pregnancy tests as simply “positive” or “negative.” A more useful report would read as follows: “Results (positive or negative). This early pregnancy test has a sensitivity of ( %) and a specificity of ( %) when the patient's beta-hCG concentration is greater than (the test's claimed analytical sensitivity).”

Although early pregnancy tests have proved helpful in the management of ectopic pregnancies, none of the four tests evaluated here is ideal and will not establish the presence or absence of early pregnancy with certainty. The results of any of these tests must be interpreted in light of these limitations.

The manufacturers of the four serum pregnancy tests evaluated here graciously donated the reagents used in this study.

References