An Algorithm for Testing and Reporting Serum Chorionic Gonadotropin at Clinically Significant Decision Levels with Use of “Pregnancy Test” Reagents

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We present an algorithm for monitoring the concentration of human chorionic gonadotropin (hCG) in serum at various clinical decision levels with use of fast, simple, and cost-effective qualitative pregnancy test reagents for hCG in serum. Based on correlation between laboratory data and clinical observations described in the literature, the following concentrations of hCG in serum can be considered as clinically significant decision levels: 5 int. units/L to exclude or “rule out” the presence of increased hCG; 25 int. units/L for “confirming pregnancy” or confirming the presence of increased hCG from sources other than the trophoblast; and 6500 and 82 500 int. units/L to enhance the sonographic diagnoses of ectopic pregnancies and molar pregnancies, respectively.

We used Tandem Icon II (Hybritech) pregnancy test reagents and evaluated the reagents for possible “false-positive” findings at the 25 int. units/L limit of hCG detectability by analyzing 100 post-menopausal and more than 4000 pre-menopausal serum specimens. The performance of the reagents was validated also at 5 int. units/L and at limits of hCG detectability >25 int. units/L.

Additional Keyphrases: ectopic pregnancy • hydatidiform mole • enzyme-linked immunosorbent assay • “kit” methods

The availability of highly sensitive and specific quantitative methods for human chorionic gonadotropin (hCG) changed the old routine of checking for pregnancy by determining hCG in serum only after a woman’s first missed menstrual period. The high analytical and clinical sensitivities of these tests have led to the new testing philosophy of “earlier is safer” and to numerous new clinical applications of hCG determinations (1).

Rapid developments in immunochemical techniques have substantially increased the diagnostic specificity and analytical sensitivity of commercially available quantitative hCG pregnancy test reagents. The analytical time required for estimating low concentrations of hCG (25 int. units/L) has also been decreased remarkably. The introduction of enzyme-linked immunosorbent assays (ELISA) in combination with two-site "sandwich" techniques has eliminated the need for radioactive detectors and has allowed reliable detection of low concentrations of hCG directly in the emergency room, at the bedside, or in the physician’s office, without extensive specialized training and equipment.

Despite the fact that several different concentration values of hCG are clinically meaningful in making differential diagnoses (1–26) (see Results and Discussion) and despite the significantly improved accuracy and reliability of “qualitative” hCG determinations, the use of the modern pregnancy test reagents is, in general, still directed at providing information for only one predetermined hCG concentration, which is deemed appropriate to demonstrate the existence of pregnancy.

To make maximum use of the new simple, fast, and cost-effective “qualitative” pregnancy tests to provide the most relevant information for each of the numerous different clinical uses, one should be able to use the pregnancy test reagents not just to estimate one hCG concentration (i.e., 25 int. units/L) to confirm pregnancy, but also to monitor the hCG concentration at any other clinically significant or critical hCG decision levels (26). This would be especially appropriate if the patient is clinically unstable and (or) a quantitative hCG method is not readily available. For clinically stable patients a quantitative hCG method would probably be the method of choice for some of these applications.

Here we demonstrate some ways that pregnancy test reagents, with suitably adjusted limits of hCG detectability, could appropriately be used to replace the more time-consuming and more expensive quantitative hCG methods for use in carefully selected cases.

Materials and Methods

Reagents

We obtained Tandem Icon II (serum) pregnancy test reagent kits for qualitative assays of serum hCG from Hybritech Inc., San Diego, CA 92121. This immunoenzymatic method, based on modified “sandwich” techniques, is calibrated against the WHO First International Preparation (1st IRP) for hCG. Unless otherwise stated, we express all hCG concentrations in units of the 1st IRP. One ampule of the 1st IRP (batch no. 75/537) contains 70 μg of highly purified hCG, which was determined to be 650 int. units (9).

The Tandem Icon II kit differs from its predecessor, the Tandem Icon kit (3, 4), in two ways. The Tandem Icon II has, in addition to the Test Zone, a built-in Positive Reference Zone and a Negative Control Zone. The color produced at the Positive Reference Zone is equivalent to the color produced by an unknown sample that contains 25 int. units of hCG per liter. The Positive Reference Zone serves as an internal assay control: it turns blue only if the assay was performed correctly and the reagents functioned properly. In addition, the Positive Reference Zone serves as a reference standard to which the color of the Test Zone can be compared, thereby setting the assay detection limit to 25 int. units/L (1st IRP). The Negative Control Zone provides a built-in quality control for the wash step and for immunological interference. If the Negative Control Zone shows any blue color, then the assay is not valid.

Setting the Limits for hCG Detection with the Icon II

The manufacturer states in the package insert that the limit of hCG detectability of the Test Zone is 10 int. units/L, that is, the kit will consistently detect serum hCG concentrations ≥10 int. units/L. The standard protocol calls for assay of 0.5 mL of serum. When we assayed 0.5 mL and
considered any blue color that appeared in the Test Zone as a positive finding, then the assay's limit of detectability was 10 int. units of hCG (1st IRP) per liter. When we used a 1.0-ml sample and considered any blue color in the Test Zone to be positive, then the limit of detectability was 5 int. units/L. This latter limit was used for the tests intended to "rule out" the possibility of pregnancy.

The limit of detectability of the assay for hCG can be set to 25 int. units/L by comparing the color of the Test Zone with that of the Positive Reference Zone. Therefore, if only those specimens were interpreted as positive for which the color of the Test Zone was equal to or darker than that of the Positive Reference Zone, then the limit of detectability was 25 int. units/L. We used this cutoff value for hCG detectability for the "confirming" pregnancy test and for specimens diluted 260- or 4000-fold with isotonic saline before assay with the Icon II. For the latter two cases the overall cutoff values for positive results were 6500 and 100 000 int. units/L (1st IRP), respectively.

Assessing the Precision of Interpreting the Icon II Color

The quality-control materials provided by most manufacturers for qualitative ELISA or RIA methods have hCG concentrations too high to be useful with a sensitive procedure for monitoring the assay performance. For example, with the Icon II, the dark color produced by 100 int. units/L of hCG would remain positive and unchanged in the presence of subtle changes in reagent activity and color development.

To monitor the integrity of the reagents, the lot-to-lot precision of color development, and the precision of interpreting the color of Icon II with a sensitive method, we created an "in-house" serum pool from specimens received in the laboratory for routine pregnancy testing. The hCG in the in-house serum pool was titrated with hCG-free serum from men to yield a test result color that as nearly as possible matched the color of the Positive Reference. Quantitative determinations (MAIA kit; Serono Diagnostics, Inc., Randolph, MA 02368) showed that the hCG concentration of this pool was 25 int. units/L, an indication that the sensitivity of the two methods is similar. We stored this serum pool in 1-ml aliquots at -20°C. Each day of this study and each day during the routine use of Icon II, we analyzed one aliquot from the pool to monitor the precision of reagent performance and the technologist-to-technologist interobserver precision of interpretation of the results. The results for the in-house pool were interpreted according to whether the color of the Test Zone was equal to, less than, or more than that of the Positive Reference.

Evaluating for "False-Positive" Results

To evaluate the Icon II for false-positive findings at the 25 int. units/L cutoff value for hCG detectability, we analyzed 100 serum samples from post-menopausal women and more than 4000 samples from pre-menopausal women, using procedures described earlier (4). The 4000 serum samples from women of childbearing years that we tested with the Tandem Icon II had been sent to our laboratories for pregnancy testing. The collection and testing of these samples spanned more than 25 months and involved many different lots of the reagent kit.

Each specimen was tested at least once, exactly according to the manufacturer's instructions. In addition, the specimens were also tested at cutoff values for hCG detectability other than that recommended by the manufacturer by increasing the sample volume or by diluting the patients' specimens with isotonic saline (see above). All other conditions remained exactly as described by the manufacturer.

To minimize the effect of technologists' subjectivity, two technologists interpreted each potentially ambiguous ELISA test result where the color of the Test Zone was so slight that the first technologist was not certain whether the result was negative or positive. If the second technologist was also uncertain about the result, or found the result to be negative, then the result was reported as negative. In this case, two persons found the result to be not positive. If the second technologist had found the result to be positive, then the result would have been judged as indeterminate, but this did not occur. In our laboratory, similar "indeterminate" results of routine "confirm" pregnancy testing are reported as "negative" with a comment that "a repeat test is suggested after 48 to 72 h."

The hospital medical records of post-menopausal patients whose serum specimens gave positive pregnancy test results at the hCG cutoff values of 10 int. units/L were examined for relevant information such as clinical diagnosis, presence or absence of malignancies, and drugs.

Validating Reagent Performance at Altered Limits of hCG Detectability

We validated the analytical limits of hCG detectability of the Icon II reagents with serum volumes >0.5 mL as described earlier (4). In brief, we added 2-mL samples of serum containing about 6 int. units of hCG per liter to the Icon II reagents. The color that developed was indistinguishable from the color of those tests in which 0.5 mL of the 25 int. units/L samples was used.

Validating the Dilution of Specimens with Isotonic Saline

To validate the use of isotonic saline as diluent for samples containing hCG >25 int. units/L, we created 20 9-mL hCG-positive serum pools from specimens submitted to our laboratory for routine pregnancy testing. Each of these pools was subdivided into three 3-mL aliquots and stored at -20°C. The first aliquot of each pool was used for quantitative determination of hCG (by MAIA, calibrated against the 1st IRP). The hCG concentration of these samples varied from 700 to 100 000 int. units/L. The second aliquot of each pool was diluted with isotonic saline to obtain a final hCG concentration of 25 int. units/L as follows: we added 25 μL of the aliquot to a volume of the diluent, using 1 mL of diluent for each 1000 int. units/L increment of hCG determined by the quantitative results for the original hCG concentration. To test the hCG in a serum pool having 6500 int. units/L, we added 25 μL of the serum pool to 6.5 mL of sodium chloride solution. After dilution, each pre-diluted sample was tested by the Icon II reagents exactly according to the manufacturer's instructions. The third aliquot of the frozen pools was used for repeat tests in case errors were suspected in the results.

Results and Discussion

Several clinical conditions are associated with low hCG—ectopic pregnancy, undiagnosed abortion, blighted ovum, and "ruling out" early pregnancies for patients who are awaiting surgery, radiotherapy, menstrual regulation, or sterilization—as it is desirable to select the most sensitive pregnancy test for "around-the-clock" routine use.

More-sensitive hCG detection inherently leads to loss of analytical specificity. Thus it is critical that the new, highly
sensitive reagents are thoroughly evaluated for "false-positive" results (2-4). Reports of "discordant," "aberrant," and "false-positive" hCG findings were summarized and the possible causes were discussed (3). The critical need to obviate false-positive findings is underlined by the following. (a) The analytical limit of hCG detectability of the recently introduced qualitative hCG assays (25-50 int. units/L) is approaching the hCG concentration of the reported discordant hCG results (5-40 int. units/L) that were found by quantitative hCG assays (2-4); (b) currently, there are no practical and effective methods for differentiating, during routine pregnancy testing, low-titer aberrant or false-positive findings from early or ectopic pregnancies; and (c) positive results for a qualitative hCG pregnancy test often lead to or contribute to clinical decisions.

In previous communications, we reported our evaluations of the urine and serum Tandem Icon reagents (3,4), the predecessors of Icon II. Here, we describe some of the characteristics of Tandem Icon II.

Analytical Variables

**Precision.** The daily use of the "in-house" quality control serum pools shows that the day-to-day and lot-to-lot precision of Icon II color development is remarkably good. Each of the six participating technologists found, day after day and month after month, that the color of the "in-house" control was equal to that of the Positive Reference. The same data also indicate that the run-to-run interobserver agreement was better than 99%. Only on two occasions was the color of the "in-house" control less than that of the Positive Reference. The interpretation of the technologists was again in agreement. The problem was caused by defective reagents—most likely damaged in shipment, because a new shipment of the same lot of Icon II reagents gave the previous intensity of color for the "in-house" control.

The precision of interpreting the color of unknown samples at 6500 and at 100 000 int. units of hCG per liter can be expected to be the same or better than that of the "in-house-control," because: (a) After appropriate dilutions, the hCG concentration would be the same, in both cases, as that of the "in-house control" (25 int. units/L). If the hCG concentration of the patients' specimens was >6500 or >100 000 int. units/L, respectively, then the interpretation would be even easier because the color of the diluted unknown would be darker than that of the Positive Reference. (b) A 9 g/L aqueous NaCl solution was found to be an acceptable diluent (see below).

Gochis et al. (26) found the interobserver agreement in interpreting the Icon II to be 98.2% when the technologists did not have knowledge of the hCG concentration in similarly diluted samples.

**Specificity.** The use of specimens from post-menopausal hospitalized women (ages >55 y) for testing for false-positive results facilitated the screening for interference from a wide variety of diseases, pituitary hormones, and drugs and, at the same time, simplified the interpretation of results. The use of specimens from post-menopausal women allowed us to exclude the possibilities of "subclinical pregnancies"—some of which may not be possible to document—from our analysis, because these occur far less frequently in post-menopausal than in pre-menopausal women. We applied such an approach in a previous study (2) to adjust the limit of hCG detectability of a qualitative RIA for urine hCG so as to avoid "false-positive" results. During the following three years, the routine use of the adjusted RIA pregnancy test for testing specimens from women of childbearing years indicated that the experimental design and the selection of urine specimens appeared to be valid for effective screening of pregnancy test methods to avoid "false-positive" results.

Table 1 summarizes the results of our evaluation of Icon II for "false-positive" results. We found no specimens from post-menopausal women that had hCG concentrations ≥25 int. units/L. We found five and seven post-menopausal serum specimens that gave positive results with the Icon II reagents at sensitivities of 10 and 5 int. units/L hCG, respectively.

Further to increase the specificity of our study, we examined the medical records of patients whose serum samples gave positive results at the 10 int. units/L limit of hCG detectability, looking for information that would explain the positive results. These patients' illnesses are summarized in Table 1.

Even though the medical records of these patients did not evidence the presence of either trophoblast or other hCG-secreting tissues, these results cannot be interpreted as "false-positive," because the presence of hCG was confirmed by a quantitative hCG procedure (see Table 1). Furthermore, the presence of low hCG concentrations in the blood of post-menopausal women has also been reported by others (6).

A further indication of the high specificity of the Icon II reagents is the following: These reagents were used for over 25 months at the limit of detectability of 25 int. units/L (1st IRP) hCG for routine pregnancy testing of more than 4000 pre-menopausal serum specimens. The staff has not reported any suspected "false-positive" findings to us.

**Validating the use of isotonic saline as diluent of specimens.** When serum samples containing hCG >25 int. units/L were diluted with isotonic saline to yield an hCG concentration of 25 int. units/L, the color developed by 18 of the 20 samples was indistinguishable from the 25 int. units/L Positive Reference Zone. These data indicate that (a) the sensitivities of the quantitative method and that of the Icon II were very similar and that (b) predilution of the samples with sodium chloride solution was an acceptable procedure.

The remaining two samples gave slightly darker colors than did the Icon II Positive Reference Zone, indicating errors in the results of either one or both of the two methods, or errors in the predilution of the samples. Frozen aliquots of these two samples were retested by both methods. When diluted according to the second quantitative results, the color of the Icon II Test Zone of these two samples was also

### Table 1. Clinical Descriptions of Post-Menopausal Patients with hCG-Positive Results

<table>
<thead>
<tr>
<th>hCG, int. units/L</th>
<th>Tandem Icon II</th>
<th>Quantitative method</th>
<th>Case histories</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10&lt;25</td>
<td>14</td>
<td></td>
<td>55 y, squamous-cell carcinoma of the lung, hypertension</td>
</tr>
<tr>
<td>&gt;10&lt;25</td>
<td>11</td>
<td></td>
<td>64 y, diabetes mellitus, cerebellar atrophy</td>
</tr>
<tr>
<td>&gt;10&lt;25</td>
<td>9</td>
<td></td>
<td>75 y, aortic stenosis, bilateral cataracts</td>
</tr>
<tr>
<td>&gt;10&lt;25</td>
<td>8</td>
<td></td>
<td>60 y, alcoholic cirrhosis, type II diabetes mellitus</td>
</tr>
<tr>
<td>&gt;10&lt;25</td>
<td>-</td>
<td></td>
<td>63 y, obesity, phlebitis, depression</td>
</tr>
</tbody>
</table>

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identical to the 25 int. units/L Positive Reference. These data suggest that the contradiction in the first set of results was caused by the quantitative procedure. The errors probably were caused by random variability, amplified by the dilution factors during the calculation of the final results.

Monitoring hCG Concentrations at Clinical Decision Levels

Published laboratory and clinical data (1–26) suggest that current clinically significant hCG decision levels include the following:

- 5 int. units/L to "rule out pregnancy" (5)
- 25 int. units/L to "confirm pregnancy" (1–15)
- 6000–6500 int. units/L, a "discriminatory zone" to enhance the accuracy of diagnosis of ectopic pregnancy by sonography (17–21, 26).
- 85 000 int. units/L, to enhance the accuracy of diagnosis of molar pregnancy by sonography (25).

The hCG concentration of 25 000 int. units/L has been implicated to enhance the accuracy of "ruling out" molar pregnancy by sonography, but the existing data are not yet sufficiently firm. Should these findings be validated, testing at a decision level of 25 000 int. units/L hCG can then be added to the algorithm presented in this communication.

The algorithm in Figure 1 depicts the way qualitative serum hCG "pregnancy test" reagents could be used to determine whether the serum hCG is less or greater than the above-listed clinically significant decision levels for hCG. The procedures described in this communication are based on the Imm II reagents, but any other appropriate pregnancy test, if properly validated, could be used to achieve the same objectives. A reagent that could be applied for the described procedures must meet the following three criteria: (a) It must not yield "false-positive" results at the limit of hCG detectability that is used for "confirming pregnancy." A reagent with a lower limit of hCG detectability (higher sensitivity) for "confirming pregnancy" provides a more efficient performance. (b) It must be possible to lower the limit of hCG detectability of the reagent to about 5 int. units/L, either by use of higher volume of unknowns or by the use of a 5 int. units/L reference standard. (c) The reagent should accept an inexpensive solution (e.g., isotonic saline) as a diluent of unknown samples to adjust the limit of hCG detectability above the level established by the manufacturer (25 int. units/L for the Imm II reagents).

The following paragraphs are numbered to facilitate reference to the algorithm presented in Figure 1. The numbers of the paragraphs correspond to the numbers in the algorithm.

1. "Rule out pregnancy." Low concentrations of hCG (<25 int. units/L) could exist in early or abnormal pregnancies, so the hCG concentration that is generally monitored for confirming pregnancies, even with the more modern pregnancy tests (25 int. units/L), is too high to effectively eliminate the possibility of pregnancy. A higher degree of certainty is desired to "rule out pregnancy" in case the patient is "at risk" of being pregnant (i.e., patients who are awaiting surgery, radiotherapy, roentgenography, menstrual regulation, or sterilization, and when ectopic pregnancy is suspected). A pregnancy test reagent that can test for hCG at the level of 5 int. units/L could be used to reach this objective.

2. A negative finding for hCG at 5 int. units/L 1st IRP "rules out" pregnancy, or much decreases the possibility of hCG-secreting tissues being present, if a pregnancy is defined as the "existence of an implanted, dividing, fertilized ovum" (5).

3. A positive finding obtained with a semiquantitative hCG assay at 5 int. units/L cutoff value for hCG detectability cannot be used as a reliable indicator of pregnancy, because low concentrations (5–25 int. units/L) of hCG are

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Fig. 1. Algorithm for use of qualitative assays of serum hCG ("pregnancy tests")

The numbers outside of the boxed statements correspond to the numbered paragraphs in Results and Discussion

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found in the serum of nonpregnant humans (6–15). The clinical significance of the reported "discordant" or "aberrant" results is not yet known, so a positive result at 5 int. units/L needs to be followed by a test at the 25 int. units/L limit of hCG detectability. Normal pregnancy should be considered "confirmed" (if the likelihood of malignancy is small) only if the hCG concentration in the serum exceeds 25 int. units/L.

4. "Confirm pregnancy"—"routine pregnancy testing." As the name implies, the traditional and routine use of pregnancy tests is to "confirm pregnancy" already suggested by clinical history, physical findings, and other information. Currently, many highly specific pregnancy test reagents can be used to confirm pregnancy at 25 int. units/L (1st IRP) (2–4).

We found several pregnancy test reagents (3, 4) that had performance characteristics similar to the Icon II. We selected the Icon II for routine use in our laboratory because:

(a) During our evaluation it did not yield false-positive results at either 25 or at 10 int. units/L limits of hCG detectability.

(b) The limit of hCG detectability could be lowered to 5 int. units/L by using a larger sample volume. It could be used to "rule out" or "confirm" pregnancy, thus eliminating the need to maintain reagents for an additional assay.

(c) Isotonic saline solution could be used to predilute the unknown specimens when testing for hCG >25 int. units/L.

(d) The color of the reaction was stable, which eliminated the need for exact timing of the last step of the procedure. Other products, similar to the Icon and Icon II reagents, did not have this characteristic (3, 4).

(e) It does not require a gamma counter, and it could be performed without extensive training in the emergency room, at the bedside, or in physicians' offices.

5. A negative result at 25 int. units/L limit of hCG detectability does not "rule out" pregnancy with a high percent of probability, because very early or abnormal pregnancy could exist with hCG values <25 int. units/L (5). To "rule out" pregnancy more effectively, repeat the test at 5 int. units/L limit of hCG detectability or repeat the test after 48 to 72 h at 25 int. units/L limit of hCG detectability. If the results are still negative, the existence of pregnancy can be "ruled out." If the hCG concentration exceeds 5 int. units/L but is less than 25 int. units/L, then early or abnormal pregnancy or other sources of hCG might exist but not be "confirmed." Concepts outlined in paragraph 3 apply in this case also, and a repeat test is suggested after 48 to 72 h in this case as well. Quantitative determination of hCG might be considered, especially if ectopic pregnancy is suspected.

6. If the hCG concentration exceeds 25 int. units/L, then pregnancy is confirmed. To verify whether a pregnancy is normal during the first eight weeks, serial determinations of hCG by a quantitative method is recommended, to demonstrate a normal rate of increase in serum hCG concentration (at least 66% increase in 48 to 72 h) (18, 20).

7. To facilitate diagnoses of abnormal pregnancies by sonography and to "differentiate" the sources of hCG at levels higher than 25 int. units/L: By dilution of the unknown specimens before analysis, semiquantitative hCG methods can be used to determine whether the hCG titer is above or below critical hCG decision levels. Normally, a quantitative hCG method is used for such tasks. In an emergency or when quantitative hCG methods are not available, the results of a semiquantitative method could possibly be used.

8. Even though ectopic pregnancy represents 0.3% to 1% of all pregnancies and accounts for about 6.5% of maternal death in the United States, definitive diagnosis of ectopic pregnancies is still difficult (16).

The usefulness of sonography alone for the diagnosis of ectopic pregnancy is limited, because intra-uterine sac was found on ultrasound examinations in 10% to 20% of patients with ectopic pregnancy (16, 17). The combined use of intrauterine or adnexal sonography and hCG determinations, however, enhanced the accuracy of the detection of ectopic pregnancies (17–24, 26). The usefulness of monitoring the "discriminatory zone" of hCG concentration (6000–6500 int. units/L) during the first trimester, to detect ectopic pregnancies, has been described (17–21, 26). The hCG concentration was 6000 to 6500 int. units/L when the sac of a normal intrauterine pregnancy became visible by ultrasound. Only about 10% of ectopic pregnancies had hCG values >6500 int. units/L when they first presented (17).

If sonography was positive for intra-uterine sac and the hCG exceeded 6500 int. units/L, then the likelihood of ectopic pregnancy was only about 1% to 2%. Such data could be used to lower the concern for ectopic pregnancy before fetal heart activity could be demonstrated. On the other hand, if the fetal sac was not visible by sonography when the hCG was >6500 int. units/L, then the data showed good correlation with ectopic pregnancy. Such a constellation of data could be used to increase the suspicion of ectopic pregnancy (17, 24).

The absence of an intra-uterine sac at hCG below the "discriminatory zone" could not be considered as a diagnostic finding, and could be used neither to increase or decrease the suspicion of ectopic pregnancy. In contrast, if intrauterine sac was found by sonography at hCG concentrations below the "discriminatory zone," then the data correlated with abnormal pregnancy, either a missed abortion or an ectopic gestation (17, 24).

To monitor the "discriminatory zone" by a semiquantitative pregnancy test reagent, a limit of hCG detectability of 6500 or 5000 int. units/L might be appropriate (26). If the limit of hCG detectability of the pregnancy test reagents is 25 int. units/L, then the unknown specimens should be diluted 260-fold to change the limit of detectability to 6500 int. units/L. In the case of the Icon II, the hCG concentration in the original unknown is judged to be positive if hCG ≥6500 int. units/L if the color density of the Test Zone of the 260-fold diluted specimen is equal to or greater than that of the Positive Reference.

While this communication was being prepared, Gochis et al. (25) also described the use of Icon II for detection of ectopic pregnancies by monitoring the "discriminatory zone" of hCG concentrations in conjunction with sonography. They diluted the patients' specimens 260-fold with hCG-free serum before assay with the Icon II. The dilution converted the cutoff value for hCG detectability of the assay to 5000 int. units of hCG per liter. The clinical false-positive rate for the modified Icon II was 6%. The value of serial quantitative hCG determinations in diagnosing ectopic pregnancies has also been described (20, 24).

9. Because there is an overlap between the hCG titers of normal pregnancies and hydatidiform moles, the determinations of hCG in serum or urine alone for diagnosis of gestational trophoblast are of limited value, especially during the late first and early second trimesters (25). The diagnosis of hydatidiform moles by ultrasound alone during the first trimester is also difficult, because the vesicles are
to small to be resolved by ultrasound. In contrast, the combined use of hCG determinations and ultrasound have been shown to increase the specificity of diagnosis by sonography. It has been suggested that hCG titers under 25,000 int. units/L, when coupled with normal intra-uterine sac, make the diagnosis of hydatidiform mole unlikely (25).

If values are between 25,000 and 85,000 int. units of hCG per liter, the presence of an intra-uterine sac in the absence of other sonographic findings may be associated with normal or abnormal pregnancies. In these cases, repeated tests are recommended to make the differential diagnosis. Above 82,350 int. units/L, the failure to observe a pulsating heart by ultrasound permitted the identification of patients at risk for gestational trophoblastic disease in the first trimester (25). To monitor hCG >82,350 int. units/L by a semi-quantitative pregnancy test reagent, we chose a limit of hCG detectability of 100,000 int. units/L, so as to simplify the scheme of dilutions of the specimen (4000-fold) before assay.

10. If sonography is not available, then serial determination of hCG at about three-day intervals could be used to determine whether the hCG concentration is stable or declining. A titer of hCG that was declining from >100,000 int. units/L to lower values was consistent with normal pregnancy.

At the University of Texas Medical Branch, after consultations with the staff of the Department of Obstetrics & Gynecology, the clinical chemistry laboratory is providing pregnancy tests at three different sensitivities 24 h a day, seven days a week. All three tests are appearing on the request forms as: "Confirm Pregnancy Test" (25 int. units/L hCG limit of detectability), "Rule Out Pregnancy Test" (5 int. units/L hCG limit of detectability), and "Ectopic Pregnancy Test" (6500 int. units/L hCG limit of detectability).

Conclusions

The Tandem Ion II reagents are applicable to monitoring multiple clinically significant decision levels for hCG in addition to the 25 int. units/L hCG recommended by the manufacturer to "confirm" existence of pregnancy. The enhanced utilization of an appropriate qualitative hCG pregnancy test reagent offers a practical and effective alternative to the use of the far more burdensome and expensive quantitative hCG procedures. These fast and cost-effective qualitative or semiquantitative methods are especially useful in an emergency when the condition of the patient mandates rapid turnaround time and a quantitative hCG method is not applicable or available. If the patient is clinically stable and a quantitative hCG method is available, then a quantitative hCG method could be the method of choice to use with the algorithm described above.

The hCG decision levels of "ruling out" and "confirming" pregnancy are based on clearly formulated clinical needs and analytical principles. The exact limits of hCG detectability used to monitor these decision levels depend mostly on the analytical and clinical specificity of the pregnancy test reagents selected. Currently, state-of-the-art limits of detectability for these hCG decision levels are 5 and 25 int. units/L, respectively. With further experience and improvements in the technology of qualitative hCG determinations, both of these limits of detectability could be revised in the future.

If pregnancy was already suspected from other available information, then it would probably be more efficient to order first a "confirm" pregnancy test and perhaps do the "rule-out" test only if the "confirm" test was negative. However, if pregnancy was not suspected, but ruling out pregnancy would still be desired (i.e., patients awaiting surgery, radiotherapy, roentgenography, menstrual regulation, or sterilization), then ordering the rule-out test first could be more appropriate. In this case, the confirm test would need to be done only if the rule-out test was positive.

The exact limits of hCG detectability to be used for evaluating abnormal pregnancies are somewhat less well defined. Because these clinical hCG decision levels are used in conjunction with sonography, the hCG "discriminatory zone" may vary depending more on the sonographic equipment used, the technique of sonography, the expertise of the sonographer, and the clinical judgement and experience of the attending physician than on the analytical principles of hCG testing. For detection of ectopic pregnancies, Kadar, Romero, and coworkers (17–19, 21, 24) suggested the use of 6000 to 6500 int. units/L hCG (1st IRP) "discriminatory zone," but Nyberg et al. (19, 22, 23) recommended 1800 int. units/L (2nd IS) or about 3600 int. units/L (1st IRP). The algorithm presented in this communication could be adjusted to any other clinical decision levels preferred by individual clinicians. To adjust the algorithm for evaluation of any other clinical hCG decision levels, the limits of hCG detectability of the qualitative hCG pregnancy tests need to be adjusted to the preference of the user. In the case of the Tandem Ion II, the limits of hCG detectability can be adjusted simply by changing the predilution of unknown specimens before the hCG analysis.

The Tandem Ion II (or other similar kit) could perhaps be used without extensive training, but the modified procedures described here should perhaps be performed only by qualified personnel who regularly perform clinical laboratory tests. Well-written protocols could aid the work of less experienced personnel.

References