Detection of Early Pregnancy Forms of Human Chorionic Gonadotropin by Home Pregnancy Test Devices

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Background: Home pregnancy testing devices claim >99% diagnostic accuracy for pregnancy and utility on the first day of the missed menses or earlier. We investigated the forms of human chorionic gonadotropin (hCG) in early pregnancy urines, the diagnostic accuracy claim, and the abilities of 15 devices to detect the different forms of hCG.

Methods: We measured the concentrations of regular hCG and hyperglycosylated hCG (H-hCG, a large hCG variant) in 592 urines. Fifteen home devices were tested according to manufacturers' instructions with regular hCG and H-hCG diluted in urine.

Results: H-hCG was the principal hCG-related molecule in pregnancy urine in the 2 weeks following the missed menses (61% and 50% of total immunoreactivity in the 4th and 5th completed weeks of pregnancy, respectively). Of 15 home test devices, 2 had a detection limit of 6.3 IU/L for regular hCG, but poorer detection of H-hCG. Two devices detected 13 IU/L regular hCG, one with similar detection and one with poorer detection of H-hCG. Ten devices detected 25 IU/L regular hCG, 6 with poorer detection of H-hCG. One device detected 50 IU/L regular hCG, but better detected H-hCG. Overall, 9 of 15 devices did not detect H-hCG as well as regular hCG.

Conclusions: H-hCG is the principal hCG immunoreactivity in early pregnancy urine. Home tests vary widely in detection limits for regular hCG (6.3–50 IU/L), and 9 of 15 devices (60%) had poorer detection limits for H-hCG than for hCG. The variation in analytical detection limits appears contradictory to the common claim for all devices of >99% detection of pregnancy on the first day of the missed menses or earlier. We suggest that manufacturers calibrate devices for both hCG and H-hCG and determine the detection rates for pregnancy rather than the proportion of positive results at arbitrary hCG concentrations.

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Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two dissimilar subunits, α and β, joined noncovalently. More than 40 years ago, the immunologic detection of hCG for diagnosis of pregnancy (1) replaced the bioassay (2). The sequence of immunologic milestones since then has taken early pregnancy detection out of the clinical laboratory and into the home. The discovery of pregnancy is often in the week following the first missed menstrual period (the 4th completed week since last menstrual period, or 4 weeks 0 days through 4 weeks 6 days), as determined by a urine home pregnancy test.

Approximately 22 home pregnancy testing devices are sold in the US. [Device list was obtained from the Food and Drug Administration (FDA) website http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm showing current approved devices, with availability of devices confirmed with manufacturers; only single listings were considered when device was listed in single, combined, and multiple packaging.] All claim “Over 99% Accuracy” or “Over 99% Accuracy in Laboratory Tests” on their packaging. Most claim “use any time of the day”, and all but one state “use as early as the day of the missed menstrual period”. One brand also claims that their test is >99% accurate and appropriate for use “three days before the expected period” or ~4 days after implantation of pregnancy.

The USA hCG Reference Service Internet site has received e-mails from women who have had puzzling...
negative urinary home pregnancy test results or negative urine test results with a positive result from quantitative serum hCG test. These may be attributable to the insensitivity of a home pregnancy test. We are also occasionally contacted by women with a positive home pregnancy test that, when repeated 2–7 days later (home pregnancy test or quantitative serum hCG test), produced negative results. These are likely attributable to early pregnancy loss, a source of increased hCG on and before the days of the missed period (3, 4). These inquiries and the queries and endeavors of Intervention, Inc. (San Francisco, CA), a California-based consumer protection group, have led us to investigate the diagnostic accuracy claims of home pregnancy tests.

Home pregnancy tests measure hCG in spot urine samples. We searched the Medline database for reports describing urine hCG concentrations in naturally fertilized pregnancies on the day of the missed menstrual period, as measured by immunometric or sandwich-type assays; none were found. We did find two large studies reporting serum hCG concentrations on or around the time of the missed menstrual period, as measured by these assays. The first, a report by Heip et al. in 1985 (5), indicated a median of 205 IU/L hCG in serum with a range of 3–7340 IU/L during the 4th completed week since last menstrual period. The second, a mathematical model by Fritz and Guo in 1987 (6) based on 272 serum samples, indicated a median of 560 IU/L and a range (5th to 95th centile) of 6–19 950 IU/L in the 4th completed week since last menstrual period. Both reports indicate a very wide range of serum hCG values for the 4th completed week since last menstrual period. Although early studies using RIAs indicated comparable hCG concentrations in serum and urine (7), more recent reports show that urine hCG concentrations are approximately one-half of, or less than one-half of corresponding serum concentrations (8–11), with significant variation occurring in serum and urine results because of diurnal variation (10, 12). The lack of publications in the past 20 years describing early pregnancy urine hCG concentrations and the extreme variability in serum hCG concentration in early pregnancy further complicate the interpretation of accuracy of pregnancy test devices.

Recent studies show that hyperglycosylated hCG (H-hCG), a large variant of hCG (M, 41 000–42 000 vs 36 000–37 000) with notably larger oligosaccharide side chains, is the principal form of hCG produced in the week following implantation (>80% of hCG immunoreactivity) (13). Additional studies of naturally fertilized and in vitro-fertilized pregnancies indicated that H-hCG may also be the principal source of hCG immunoreactivity in serum and urine at the time of the missed menstrual period (8). We investigated the proportions of hCG and H-hCG present in urine in naturally fertilized pregnancies throughout gestation. We also examined the detection limits of 15 home pregnancy test devices to detect hCG and H-hCG. We considered the results of these studies, the published data on early pregnancy hCG concentrations and on early pregnancy losses, and the appropriateness and validity of manufacturers’ accuracy statements for home pregnancy devices.

Materials and Methods

Urine samples

Urine samples were obtained at different times of the day (during working hours) from pregnant patients attending maternity and maternal fetal medicine clinics for obstetric care and for prenatal screening (9). All samples were from naturally fertilized singleton pregnancies with term delivery. A total of 592 samples were collected, 218 from the first, 289 from the second, and 85 from the third trimester of pregnancy. Samples were coded and collected in compliance with university Institutional Review Board guidelines.

Measurement of all-hCG and H-hCG in urine samples

Separate immunometric assays were used to test the 592 urine samples for both all forms of hCG dimer (all-hCG assay) and for H-hCG only (9, 14, 15). Both tests are two-step semiautomated microradiot plate assays with horseradish peroxidase-labeled tracers and computerized microradiot plate readings and calculations. The all-hCG assay uses a monoclonal anti-α-subunit capture antibody (antibody 2119; gift from Unipath Inc., Bedford, United Kingdom) and a commercial monoclonal anti-β-subunit tracer antibody (antibody 4001; Genzyme Diagnostics, San Carlos, CA). The all-hCG test detects regular hCG, regular nicked hCG, and H-hCG (9, 15). The test for H-hCG only uses a specific monoclonal against H-hCG (B152) as capture antibody (14, 15) and the same commercial anti-β-subunit (4001) tracer. This test detects only H-hCG (9, 15). Standards, pure hCG CR127 (gift of S. Birken, Columbia University, New York, NY), and pure H-hCG type C5 [purified from choriocarcinoma patient urine (16)] were calibrated by mass based on amino acid analysis. For 3 years, our all-hCG assay has included 1st International Reference Preparation [IRP; 3rd International Standard (IS)] standards to determine IU. Consistently, 11 IU/L regular hCG has been equivalent to 1 μg/L (15). Results from all-hCG assays were converted to IU, based on this value (14). There is no international standard for H-hCG. H-hCG was calibrated by the all-hCG assay using both mass and 1st IRP (3rd IS). We observed a mass-to-IU ratio for H-hCG similar to that for regular hCG immunoreactivity (11 IU/L = 1 μg/L) and used that ratio to calculate H-hCG IU values (14, 15). Urine creatinine was determined by the picric acid method, using the chemistry reagent set and creatinine standards produced by Sigma (cat. no. 555A), adapted for microradiot plate applications (9, 14, 15). All-hCG and H-hCG only concentrations are expressed as IU/g of creatinine.
HOME PREGNANCY TESTS

In a search of 10 major drug stores chains and pharmacies in the Albuquerque and San Francisco areas, we were able to purchase 15 of the ~22 available home pregnancy tests. An average of 28 copies of each home pregnancy test were purchased. The devices were as follows: Answer and First Response Early Result, which are produced by Carter-Wallace Inc. (New York, NY); American Fare Easy to Read and Longs Pregnancy Test, which are produced under contract by Perrigo Co. (Allegan MI); Clear Blue Easy, which is manufactured in the United Kingdom for Unipath Diagnostic Co. (Princeton, NJ); Confirm, which is manufactured in the United Kingdom for Durex Consumer Products, Inc. (Norcross, GA); E.P.T., which is produced by Warner-Lambert Consumer Healthcare (Morris Plains, NJ); Equate, which is produced by LifeCare Medical International Corp. (Philadelphia, PA); Fact Plus Pro and Fact Plus Select, which are produced for Abbott Laboratories by Medisense Products (Bedford, MA); Inverness Early Pregnancy Test, Rite Aid One Step, and Walgreens One Step, which are produced by Inverness Medical Inc. (Waltham, MA); Target Early Pregnancy Test, which is produced by Dayton Hudson Corp. (Minneapolis, MN); and Walgreens Early Pregnancy Test, which is produced in Ireland under contract for Walgreens Co. (Deerfield, IL).

Devices were tested blindly, one product at a time, precisely following the manufacturers' instructions. Each device was tested with a coded urine sample containing different concentrations of pure regular hCG, H-hCG, or nicked hCG (5-mL aliquot in each evaluation tube) as described below. One laboratory scientist tested, and a second scientist timed each device. Each device was observed until a positive response occurred or until the recommended reading time as defined in the instruction sheet. The Equate device was examined at the recommended initial read time (1 min). Only with this device was a range of read times of 1–10 min indicated.

EVALUATION OF HOME PREGNANCY TESTS WITH URINE hCG AND H-hCG STANDARDS

Three standards were tested: regular hCG standard batch CR127 (19% nicked, containing 19% hyperglycosylated O-linked oligosaccharides; virtually identical to preparation CR119 the 1st IRP/3rd IS); H-hCG standard C5 (>90% nicked, containing 100% hyperglycosylated O-linked oligosaccharides (16]); and nicked hCG standard M4 (>90% nicked, containing 7% hyperglycosylated oligosaccharides (16]). The regular hCG, nicked hCG, and H-hCG were calibrated by amino acid analysis and then converted to IU based on the 1st IRP/3rd IS formula described above (1 μg = 11 IU). Samples were prepared by diluting high concentrations of standards (>1 g/L or >11 MIU/L) in pooled urine from 10 healthy males. For regular hCG and H-hCG, 5-mL aliquots were prepared at 50, 25, 13, and 6.3 IU/L. For nicked hCG, only 50 IU/L aliquots were prepared. All urine aliquots were mixed and blindly coded before testing. Devices were initially examined with the multiple concentrations of standards. When results were observed that were inconsistent with the dose–response trend, measurements were repeated three additional times at each point (at least three consistent values) to either confirm or correct results.

RESULTS

PROPORTION OF hCG IMMUNOREACTIVITY ATTRIBUTABLE TO H-hCG DURING PREGNANCY

All-hCG and H-hCG only were measured in 592 normal pregnancy urine samples from 4 to 43 weeks since last menstrual period (Fig. 1). Means are shown for the H-hCG contribution to all-hCG immunoreactivity for each completed week since last menstrual period (Fig. 1). All-hCG comprised 61% ± 9% (SE) of immunoreactivity in the 4th completed week since last menstrual period (n = 33; all-hCG results, 12–2538 IU/L; median, 210 IU/L; H-hCG results, 3–809 IU/L; median, 134 IU/L). H-hCG comprised 50% ± 4% in the 5th completed week of pregnancy (n = 41; all-hCG results, 13–6046 IU/L; median, 530 IU/L; H-hCG results, 8–3478 IU/L; median, 231 IU/L). The proportion of H-hCG decreased during the remainder of the first trimester (<25% of all-hCG) and declined much further through the second and third

Fig. 1. Concentration of all-hCG (●) and of H-hCG only (○) in 592 urine samples from individuals at 4–42 complete weeks since last menstrual period.

Spot urine concentrations are normalized to creatinine concentration. The median proportion of H-hCG (H-hCG ÷ hCG) was determined for each complete week since last menstrual period (X). Medians were best fit by a third-order logarithmic curve (—–).
trimesters of pregnancy (<5% of all-hCG). The five samples from the day of missed menstrual period yielded hCG results of 12, 32, 38, 116, and 356 IU/L.

**HOME PREGNANCY TEST DEVICES**

Devices were evaluated with blinded samples and read as described in the manufacturers’ instructions. Devices were first read at the recommended reading time as defined in the instruction sheet (Equate was read at 1 min; Table 1). As shown in Table 1, only 2 of the 15 devices were positive at the recommended reading time for the 6.3 IU/L regular hCG standard; two additional devices detected as low as the 13 IU/L standard, and another 10 devices best detected the 25 IU/L standard. The remaining device detected only the 50 IU/L regular hCG standard. Devices variably detected H-hCG. Two devices, E.P.T. and Confirm, detected H-hCG with lower detection limits than for regular hCG (13 vs 25 IU/L and 13 vs 50 IU/L, respectively). Four devices had similar detection limits for the two forms of hCG (American Fare Easy to Read, Answer, FactPlus Pro, and FactPlus Select), but nine devices, or the majority, had poorer detection limits for H-hCG or failed to detect any of the H-hCG standards (Table 1).

The 50 IU/L regular hCG and H-hCG standards, we considered the possibility that nicking was causing the failure of devices to recognize 50 IU/L H-hCG. All 15 devices were tested with urine containing 50 IU/L M4 nicked hCG standard. All devices gave a positive result, indicating that nicked hCG was not responsible for devices failing to detect H-hCG.

**Discussion**

Home urine pregnancy test devices are the first line of pregnancy detection for many women. They are commonly used in the week after missing a menstrual period (4th completed week). With the First Response device they may be used even earlier. During the week following the missed menstrual period, serum hCG concentrations range from 3 to 19,950 IU/L (5, 6).

A survey of approved FDA 510(k) applications and of technical service agents indicated that all home pregnancy test devices have been calibrated with regular hCG and not H-hCG. The studies described here show, however, that H-hCG, a separate and different-sized molecule, is the principal source of hCG-related immunoreactivity in urine samples at the time of and during the 2 weeks following the missed menstrual period. The proportion of H-hCG molecules rapidly diminishes as pregnancy advances, dropping to <5% of the total hCG immunoreactivity in the second and third trimesters. The H-hCG findings reported here are the most extensive reported to date (n = 592). They confirm the studies of O’Connor et al. (13) and Kovalevskaya et al. (8), which showed primarily, or in some cases solely, H-hCG production before and in the 2 weeks after the missed menstrual period. Together these findings clearly show that H-hCG is the key source of hCG immunoreactivity in early pregnancy urine samples. This suggests that H-hCG is the molecule that should be optimally recognized by and used to calibrate home pregnancy tests.

| Table 1. Evaluation of 15 home pregnancy tests with regular hCG and H-hCG standards.* |
|---------------------------------|---------|---------|---------|---------|---------|---------|---------|
| Device                          | Read at min | **Regular hCG, IU/L** | **H-hCG, IU/L** | Manufacturer’s hCG limit, IU/L |
|                                 |          | 6.3     | 13      | 25      | 50      | 6.3     | 13      | 25      | 50      |
| Clear Blue Easy                 | 1        | +       | +       | ++      | ++      | –       | –       | –       | –       | 50       |
| Target Early Pregnancy Test     | 5        | ±       | +       | ++      | ++      | –       | –       | –       | –       | 50       |
| American Fare Easy to Read      | 3        | –       | +       | ++      | ++      | –       | +       | +       | +       | 100      |
| First Response Early Result     | 3        | –       | –       | +       | +       | –       | –       | –       | –       | –50      |
| E.P.T.                          | 3        | –       | –       | –       | –       | +       | +       | +       | +       | 40       |
| Answer                          | 2        | –       | –       | +       | +       | –       | –       | –       | –       | 100      |
| Fact Plus Pro                   | 3        | –       | –       | +       | +       | –       | –       | –       | –       | 100      |
| Fact Plus Select                | 3        | –       | –       | +       | +       | –       | –       | –       | –       | 100      |
| Equate*                         | 1        | –       | –       | +       | +       | –       | –       | –       | –       | 25       |
| Walgreens E.P.T.                | 5        | –       | –       | +       | +       | –       | –       | –       | –       | 100      |
| Walgreens One Step              | 3        | –       | –       | +       | +       | –       | –       | –       | –       | 50       |
| Inverness Medical E.P.T.        | 3        | –       | –       | +       | +       | –       | –       | –       | –       | 100      |
| Longs Pregnancy Test            | 3        | –       | –       | +       | +       | –       | –       | –       | –       | 100      |
| Rite Aid One Step               | 3        | –       | –       | –       | –       | –       | –       | –       | –       | 50       |
| Confirm                         | 2        | –       | –       | +       | +       | –       | –       | –       | –       | 25       |

*Devices were evaluated at the recommended read times. –, negative result observed; ±, exceptionally faint result observed; +, clear positive result observed; ++, a strongly positive result observed. Results are presented in order of detection limit for regular hCG (we considered a strong, a clear, or an exceptionally faint result as positive). Also shown are manufacturers’ claimed hCG detection limits.

*Initial result recorded was not consistent with dose–response trend; test repeated three times to confirm given result.

*Recommended to be read after 1 min, but within 10 min.
As reported here, home pregnancy test devices vary greatly in their analytical detection limits for regular hCG (6.3–50 IU/L). Nine of the 15 devices (60%) had less sensitivity for detecting H-hCG than for regular hCG. Because H-hCG is the sole or principal form of hCG present in urine at the time of early home pregnancy testing, its detection limit is important. When we assayed urine H-hCG preparations with 6.3, 13, 25, and 50 IU/L H-hCG, three devices gave positive results at 13 IU/L, five were positive at 25 IU/L, and three at 50 IU/L. Four of the devices failed to give a positive result with any of the standards (detection limit >50 IU/L).

All 15 devices tested carried statements such as “Over 99% Detection”, “Over 99% Detection in Laboratory Tests”, or “Clinically Trusted Results, Over 99% Accuracy” on their boxes or in their package inserts. Only 1 of the 15 devices, First Response Early Result, explained the meaning of the >99% claim, stating that “with urine samples representative of both pregnant and non-pregnant subjects, laboratory technicians obtained the correct expected result in more than 99% of the samples”. The manufacturers of the other 14 devices did not specify what over 99% accurate meant. Women who contacted the USA hCG Reference Service appeared to interpret the advertised claim to mean >99% accurate in detecting pregnancy. Some of these contacts originated from a failure of a device to detect pregnancy.

All 15 devices were approved by the FDA 510(k) procedures. The procedures call for testing against regular hCG standards (1st IRP or 3rd IS) and comparing a new device with a previously approved device (guidelines are available at http://www.fda.gov/cdrh/ode/guidance/1172.html). The guidelines recommend testing at least 100 urine specimens, either confirmed pregnancy and nonpregnancy urine samples or normal male or nonpregnant female urine samples supplemented with regular hCG. The concentration of hCG should be close to the claimed detection limit. The ability to distinguish those with and without hCG is determined. The FDA recommends “expressing the data in terms of percent accuracy, which should never exceed >99%”. The FDA 510(k) appears to be the source of the >99% detection claims. The >99% accuracy claim may have no bearing on the test detection limit for detecting pregnancy. Considering that H-hCG is the principal hCG immunoreactivity in early pregnancy urine and that most devices tested had poorer detection limits for H-hCG than for hCG, the variation in analytical detection appears contradictory to the common claim of >99% detection of pregnancy on the day of the missed menses or earlier.

Table 1 shows the manufacturers’ claimed hCG detection limits. Fourteen of fifteen devices claimed detection limits two- to eightfold higher than those determined in this study. This likely reflects, at least in part, the FDA 510(k) guidelines, which directly compare a new device with an older certified device (guidelines available at http://www.fda.gov/cdrh/ode/guidance/1172.html), and a manufacturer’s adoption of the older device’s analytical limits.

Several investigators have disputed the accuracy/reliability claims of home pregnancy test manufacturers (17–21). Most of these investigators defined accuracy as the ability to detect a given amount of hCG. Blind studies were carried out with volunteers performing their own tests, each individual carrying out a single test; accuracy/reliability values of 46–89% were reported (17–21), values very different from the >98% and >99% detection quoted for consumer testing by manufacturers.

Reports on serum hCG indicate a wide range of hCG concentrations in the 4th completed week of pregnancy (5, 6). To the best of our knowledge, no report has been published on urine hCG concentrations in naturally fertilized pregnancies during the early weeks of gestation. We tested urine hCG concentrations during the course of the 4th completed week of gestation. The range of all-hCG results in urine was 12–2438 IU/L (n = 33) compared with serum concentrations of 3–7340 and 6–19 950 IU/L at a similar time of gestation (5, 6). This is consistent with a lower range of hCG values in urine than in serum samples, with a very wide range in concentrations. The lower values and wide range in urine concentrations have been indicated in other reports (8–11). The five samples in our study from the day of missed menstrual period also varied widely in concentration (12–356 IU/L). Considering the major variability in serum and urine hCG concentrations in early pregnancy, the major differences observed in the detection limits of different home pregnancy test devices, the false results reported in the literature for consumer use of home pregnancy testing devices (17–21), and false-positive rates attributable to early pregnancy losses (3, 4), the true accuracy of home pregnancy tests for detecting pregnancy on the day of the missed menstrual period and in the week following (4th completed week) seems uncertain.

In 1986, Doshi (17) claimed that three home pregnancy test devices detected, on average, 80% of pregnancies in the 4th completed week since last menstrual period with a 32% false-positive rate. Of the 15 home pregnancy test devices used here, only one, First Response Early Result, included information regarding ability to detect pregnancy. This manufacturer claimed 86% detection of pregnancy at 1 day before the missed menstrual period. We used the phone numbers given in the instruction sheets to contact technical assistance for the other device manufacturers. None were able to provide us with any data for detecting pregnancy on the first day of missed period or any other date. Six technical assistants took our phone number and stated that a supervisor would contact us with data; none did.

In conclusion, we confirm that H-hCG is the principal source of hCG immunoreactivity in early pregnancy urines and show that only 6 of 15 home pregnancy test devices analytically detect this important molecule as
effectively as they detect regular hCG. We also show wide variance in the limit of devices for detecting regular hCG and H-hCG. The data indicate that if home pregnancy test devices were reformulated to better recognize H-hCG, they could detect pregnancy even earlier than possible at present. We query the meaning of the >99% accuracy advertised by manufacturers. Considering our H-hCG findings, together with the analytical detection limits of home pregnancy tests and the wide variability in hCG concentrations, we question the claims of home pregnancy tests. Confusing claims, such as the >99% accuracy, apparently derive from FDA guidelines. We suggest that these guidelines need to be examined. FDA regulations should include detection limits for regular hCG and H-hCG. Regulations should also incorporate measurement of clinical sensitivity for detecting pregnancy on the first day of the missed menstrual period and other set time points. Defined measures of diagnostic accuracy (e.g., clinical sensitivity and specificity) should be reported on manufacturer’s packaging and inserts rather than “over 99% accuracy”.

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References