Home and Point-of-Care Pregnancy Tests: A Review of the Technology

Joel R. L. Ehrenkranz

The earliest known pregnancy test, "If the veins within her arm beat against thy hand, thou shalt say 'she is pregnant,'" dates to 1350 BC and reflects medicine's longstanding interest in developing reliable methods to determine whether a woman is pregnant. Hippocrates in 400 BC described a method to diagnose pregnancy as follows: "Ingest honey in water: if pregnant, painful abdominal distention will result." During the middle ages, "piss-prophets" used uroscopy to diagnose pregnancy. In 1200 AD, it was believed that milk would float on top of urine from a pregnant woman. In 1500 AD, coating of an iron needle with black spots when immersed in a woman's urine was thought to indicate pregnancy. In 19th-century France, the presence of a "Kysteine pellicle" in urine was used to diagnose pregnancy.[1]

The first scientifically based pregnancy test, the A Z test, was initially described by Ascheim and Zondek in 1928. This bioassay, as well as subsequent tests introduced between 1929 and 1950, which used changes in ovarian estrogen production, weight, or blood flow; uterine, prostate, or seminal vesicle weight changes in rabbits or rats; or gamete production in toads, were based on the presence of human chorionic gonadotropin (hCG) in the urine of a pregnant woman.[2] In 1957, the use of basal body temperature, which detects the rise in progesterone that triggers a rise in core body temperature occurring with ovulation and pregnancy, was introduced as a method for the detection of pregnancy.

Starting in 1960, immunologic methods to detect hCG and hence diagnose pregnancy were introduced. The first antibody-based hCG assays used heme or latex agglutination to indicate the presence of immunoactive hCG in a pregnant woman's urine. The radioimmunoassay for hCG was introduced in 1971 and the radioreceptor hCG assay in 1974.[3] In 1976, the first home pregnancy test, based on hemagglutination inhibition, was introduced for sale in the United States. By the early 1980s, home pregnancy tests were based on an enzyme immunoassay or immunochromatography to detect hCG in urine.[4] By 1988, monoclonal antibodies were introduced into home pregnancy testing. In the 1990s, the current generation of home pregnancy tests, which use monoclonal antibodies and visual labels in an immunochromatographic format, were introduced. These widely available consumer products cost pennies to manufacture, are simple to perform, provide results in under 5 minutes, and are able to reliably indicate pregnancy as early as 15 days after ovulation or 1 day after a missed menstrual period.

Human Chorionic Gonadotropin

Biochemistry

All pregnancy tests currently in use in hospitals, physician's offices, or clinics, or sold for home use, rely on equivalent components, analytic methods, and performance and are based on the ability to detect hCG in a concentration of 25 milli-IU/ml or greater in urine. hCG is one of four glycoprotein hormones and is composed of an alpha and a beta subunit. The hCG alpha subunit is identical to the alpha subunit of luteinizing hormone (LH), follicle-stimulating hormone, and thyroid-stimulating hormone, whereas the beta subunit is unique for all four glycoprotein hormones. The proposed molecular evolution for glycoprotein hormones is thought to have occurred through ancestral gene duplication to form the alpha and beta subunits, followed by beta subunit evolution in LH, follicle-stimulating hormone, and thyroid-stimulating hormone-specific proteins. Further evolution of LH, including the loss of a termination codon in the 3'-untranslated region and addition of 30 amino acids to the C-terminal beta subunit tail, resulted in the formation of the hCG-specific beta subunit. The amino acid homology between LH and hCG accounts for the fact that both LH and hCG have identical biological activity regarding steroidogenesis, bind to the same cell surface receptor, and show substantial immunologic cross-reactivity.

From the Department of Medicine, University of Colorado Health Sciences Center, Denver, and Valley View Hospital, Glenwood Springs, Co.

Address reprint requests to Joel R. L. Ehrenkranz, 0101 Oak Ridge Drive, Aspen, CO 81611; JRLE@aol.com.

Th alpha hCG subunit is 92 amino acids long and weighs approximately 14,500 daltons; the beta subunit, which is noncovalently linked to the alpha subunit, is 145 amino acids in length, weighs 22,200 daltons, and confers on hCG its receptor and biological specificity. Intact hCG contains 11 sialic acid residues that form 2 *N*-linked oligosaccharides on the alpha subunit and four *O*-linked oligosaccharides on the beta subunit. These oligosaccharides decrease the metabolic clearance of hCG and give intact hCG a half-life of 39 hours.[5]

Physiology and Pathology

hCG is synthesized by trophoblastic tissue commencing at the time of blastocyst implantation; this occurs 6 to 9 days after conception. hCG takes over the function performed by LH, which is to stimulate progesterone production by the corpus luteum during the first 10 weeks of pregnancy. Additionally, hCG is thought to modulate maternal-fetal immune function and to stimulate the fetal gonads. In addition to its manufacture by trophoblastic tissue, hCG is also manufactured by the pituitary gland[6] and by a variety of benign gastrointestinal neoplasms. Ectopic hCG production is seen with a variety of malignancies, including trophoblastic disease and neoplasms involving the lung, breast, esophagus, stomach, small intestine, pancreas, biliary tract, colon, liver, kidney, prostate, ovary, testes, cervix, vagina, and uterus, as well as in melanoma, lymphoma, leukemia, and multiple myeloma.[7]

Metabolism

Sensitive radioimmunoassays are able to detect hCG in serum as early as 6 days postconception. Maximum hCG levels occur 8 to 9 weeks after a woman's last menstrual period. Because of its long half-life, hCG can be detected for weeks after pregnancy cessation or termination.[8]

Placental macrophages cleave the beta hCG subunit between amino acids 47 and 48 to yield nicked hCG. Nicked hCG dissociates into alpha and beta subunits with a half-life of 22 ± 5.9 hours. The degradation of nicked hCG accounts for the shortened half-life of hCG when stored *ex vivo*. The urine of a pregnant woman contains free alpha and free beta subunits, beta core fragments derived from nicked hCG, and intact hCG. The proportion of each component present varies considerably between individuals.[9,10]

Immunochemistry

As discussed above, a pregnant woman's urine will contain a mixture of intact hCG, free alpha and beta subunits, and fragments of the alpha and beta subunits that result from placental metabolism of hCG. The antibody pairs used in the manufacture of pregnancy tests differ from manufacturer to manufacturer, and the various antibody pairs used in the manufacture of pregnancy tests differ in the hCG components that are detected. Some antibody pairs detect intact hCG, whereas others detect the N and C terminals of the beta subunit. An antibody pair that measures intact hCG will not be affected by the presence of nicked hCG or nicked hCG breakdown products, whereas an antibody pair that recognizes the N and C terminals of the beta subunit will be affected by nicking and beta subunit degradation. Antibody pairs that measure intact hCG correlate best with hCG biological activity, are not affected by placental hCG metabolism or subunit degradation, and yield the lowest quantitative hCG levels. In contrast, antibody pairs that measure the concentration of intact alpha or beta subunits will also not be affected by nicking, dimer dissociation, or degradation, but can yield results that do not correlate with hCG bioactivity.[11,12]

In addition to the presence of hCG breakdown products, cross-reactivity with LH represents the other major challenge to hCG measurement that results from hCG immunochemistry. As mentioned previously, there is considerable amino acid and quaternary structure homology between the beta subunits of LH and hCG. The major epitope difference between LH and hCG consists of the 30-amino acid C-terminal tail that is unique to hCG, and problems of cross-reactivity with LH have compromised and continue to compromise the specificity of a number of pregnancy tests. Incorporation of a scavenger antibody that is unique for the beta LH subunit to sequester LH present in a urine sample helps eliminate the problem of LH cross-reactivity. Additionally, the use of a C-terminal-tail epitope-specific anti-beta hCG antibody provides increased assay sensitivity and eliminates the hook effect, *ie*, false negative results that occur with high analyte concentrations.[13]

Pregnancy Test Design and Performance

Modern pregnancy tests use an immunochromatographic format to detect the presence of hCG in a urine sample. Pregnancy tests are constructed by depositing a combination of mobile-phase murine monoclonal antibodies at the site on a nitrocellulose membrane or nylon strip where sample is applied. The antibody combination includes an anti-beta subunit antibody to which a visible particulate label such as a latex particle or colloidal metal has been covalently attached. Colloidal metals commonly used include gold, silver, carbon, and selenium. Anti-beta subunit antibodies can recognize the C-terminal or other beta-hCG subunit-specific epitopes with varying binding affinities to label hCG with a sufficient visual signal so that hCG can be detected in concentrations that range from 25 to 500,000 milli-IU/ml.[14] Also included in the mobile-phase antibody combination are anti-LH scavenger antibodies and anti-immunoglobulin antibodies to remove any potentially

interfering immunoglobulins that may be present in the sample.

At a site downstream from the origin of the membrane, a capture antibody is covalently bound to the membrane. This antibody is usually one directly against an epitope on the alpha subunit. Further downstream from the capture antibody, a control antibody is covalently attached to the membrane. This internal control antibody is usually a goat anti-mouse immunoglobulin G antibody that recognizes the mobile-phase anti-beta hCG antibody.

After the application of the antibodies, the membrane is exposed to a protein such as casein or equine immunoglobulin G to block nonspecific binding. Next, a high-capacity sump is attached to the distal end of the membrane; this serves to absorb fluid that has flowed the length of the membrane and thus to drive capillary flow along the membrane. The membrane is then placed in a plastic housing that protects the membrane and antibody components from wetting by direct sample application and allows for sample addition at the membrane origin and viewing of the results, *ie*, whether visual signal is present at the capture antibody and control antibody sites on the membrane. The enclosed membrane is then placed in a hermetically sealed pouch, which provides a shelf-life of 2 to 3 years at room temperature.

Regulatory Aspects of Pregnancy Tests

Pregnancy tests are classified as class II medical devices by the U.S. Food and Drug Administration, which means that, in addition to being manufactured in accordance with accepted good manufacturing practices, they must also conform to accepted performance standards. The first home pregnancy tests were introduced for sale before May 28, 1976, and antedate the 1976 Medical Device Amendment of the Food, Drug, and Cosmetic Act. Accordingly, new devices in the home pregnancy test category are approved for sale in the United States through the 510(k) mechanism. Pending the development of pregnancy test performance standards, the U.S. Food and Drug Administration monitors products' performance and methods of manufacture, to ensure that the tests are made in accordance with good manufacturing practices and comply with FDA labeling regulations, on a biannual basis.[15]

End-User Pregnancy Test Performance

A 1982 study showed that 24.3% of home pregnancy tests gave false negative results. By 1993, the widespread introduction of monoclonal antibody-based immunochromatographic-format home pregnancy tests showed greater than 99% precision and reproducibility and 100% sensitivity and specificity. In 1982, only 32% of test users followed test instructions. In 1993, 76% of test users read the instructions before or during home pregnancy testing.[16–20]

A comprehensive study of 27 brands of pregnancy tests sold in France in 1989 showed that only 11 of 27 brands had 100% sensitivity and specificity. Using tests with 100% sensitivity and specificity, 478 women were asked to perform home pregnancy tests with urine containing hCG in a sufficient concentration to give a positive result. Of the 478 tests performed, 230 (48%) obtained false negative results. The age, activity, or education of the individual performing the test did not account for the false negative result. Additionally, 17% of the women who obtained a false negative result acknowledged not reading the instructions, whereas 24% of the women who performed the test correctly did not read the instructions. The authors of this study concluded that poor comprehension of the instructions, particularly understanding how to visually interpret the test results, accounts for the large number of false negative test results observed.[21]

Another study of telephone calls to a toll-free customer support telephone service in 1995, based upon the sales of 125,503 self-performing immunochromatographic pregnancy tests in Canada and 606,507 in the United States, revealed that the number of calls received represented 1–7% of the number of tests shipped in the prior month. Ninety-three to ninety-eight per cent of consumer questions were answered by an automated message that repeated the test instructions. Two to seven per cent of inquiries required assistance by trained personnel. No differences in the number or nature of calls was observed between the United States and Canada. Of the calls that required a personal response, the most commonly asked question related to the confirmation of a positive test result. The next most common questions were a review of the testing procedure or the use of a variant test procedure by women who performed a pregnancy test and elected not to read the instructions. The least frequently asked questions involved the effects of potentially interfering substances. Substances consumers suspected as having the potential to interfere with home pregnancy test performance included diet pills, diuretics, oral contraceptives, fertility drugs, aspirin, antidepressants, antibiotics, and alcohol (Uveges C, *et al.* personal communication, 1996).

A recently published meta-analysis on pregnancy test performance[22] reviewed ten studies from the medical literature. Of these, five studies that evaluated 16 home pregnancy tests satisfied inclusion criteria. This retrospective review concluded that test sensitivity (97–99%) was greatest when the tests were performed by laboratory personnel. Intermediate sensitivity results (91%) were obtained when volunteers were asked to perform a pregnancy test, and the lowest sensitivity in test performance (75%) was found when patients were asked to perform

pregnancy tests using their own urine samples. The authors of this study recommend that pregnancy test kit manufacturers provide data on actual patient performance of pregnancy test, as use of volunteers appears to lead to an overestimation of the sensitivity of home pregnancy tests.

Conclusions

Methods for diagnosing pregnancy, first described 4,000 years ago, have evolved from subjective and challenging diagnostic tests performed by physicians into mass-produced consumer products that are inexpensive to produce, simple to perform, convenient, and reliable. Quantum advances in pregnancy testing have resulted from basic science advances in endocrinology and immunology, whereas incremental steps in improved pregnancy test design and use are driven by market factors, such as customer use and technical improvements in test features and materials.

The current generation of pregnancy tests have 100% sensitivity and specificity in detecting hCG at concentrations of 25 milli-IU/ml or greater and thus are able to detect pregnancy as early as 1 day after a missed menstrual period. The tests are so easy to perform and interpret that no technical expertise or training, other than reading the test instructions before performing the test, is required. Pregnancy tests sold in the United States are class II medical devices the manufacture and performance of which is monitored by the U.S. Food and Drug Administration.

It is inevitable that pregnancy tests will continue to evolve. Over the short term, tests that provide faster results and are easier to use and interpret will be developed. With advances in reproductive endocrinology, it is likely that pregnancy will be detected earlier and earlier. The ultimate pregnancy test will be able to indicate the moment that conception has occurred, cost pennies, and be foolproof to perform and interpret.

References

1. Speert H. *Obstetrics and Gynecology: A History and Iconography*. San Francisco: Norman Publishing, 1994.
2. Loraine JA. *The Clinical Application of Hormone Assay*. Edinburgh: E and S Livingston, 1958.
3. Landesman R, Saxena BB. Results of the first 1000 radioreceptor assays for the determination of human chorionic gonadotropin: a new, rapid, reliable, and sensitive pregnancy test. *Fertil Steril* 1976;27:357–368.
4. Valanis BG, Perlman CS. Home pregnancy testing kits: prevalance of use, false-negative rates, and compliance with instructions. *Am J Public Health* 1982;72:1034–1036.
5. Nisula BC, Wehmann RE. Distribution, metabolism, and excretion of human chorionic gonadotropin and its subunits in man. Segal ST 1980;231–252, Plenum Press, *Chorionic gonadotropin*, New York.
6. Hammond E, Griffin J, Odell WD. A chorionic gonadotropin-secreting human pituitary cell. *J Clin Endocrinol Metab* 1991;72:747–754.
7. Cole LA. Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites. *Clin Chem* 1997;43:2233–2243.
8. Braunstein GD, Rasor J, Danzer H, Adler D, Wade ME. Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am J Obstet Gynecol* 1976;126:678–681.
9. Birken S, Gawinowicz MA, Kardana A, Cole LA. The heterogeneity of human chorionic gonadotropin (hCG). II. Characteristics and origins of nicks in hCG reference standards. *Endocrinology* 1991;129:1551–1558.
10. Cole LA, Kardana A, Park SY, Braunstein GD. The deactivation of hCG by nicking and dissociation. *J Clin Endocrinol Metab* 1993;76:704–710.
11. Cole LA, Kardana A, Andrade-Gordon P, *et al*. The heterogeneity of human chorionic gonadotropin (hCG). III. The occurrence and biological and immunological activities of nicked hCG. *Endocrinology* 1991;129:1559–1567.
12. Cole LA, Kardana A. Discordant results in human chorionic gonadotropin assays. *Clin Chem* 1992;38:263–270.
13. Cole LA, Seifer DB, Kardana A, Braunstein GD. Selecting human chorionic gonadotrophin assays: consideration of cross-reacting molecules in first-trimester pregnancy serum and urine. *Am J Obstet Gynecol* 1993;168:1580–1586.
14. Matsuura S, Chen HC, Hodgen GD. Antibodies to the carboxyl-terminal fragment of human chorionic gonadotropin beta-subunit: characterization of antibody recognition sites using synthetic peptide analogues. *Biochemistry* 1978;17:575–580.
15. Johnson W. FDA's comments on in-home pregnancy tests. *Am J Public Health* 1986;76:588.
16. Asch RH, Asch B, Asch G, Asch M, Bray R, Rojas FJ. Performance and sensitivity of modern home pregnancy tests. *Int J Fertil* 1988;33:154, 157–158, 161.
17. Doshi ML. Accuracy of consumer performed in-home tests for early pregnancy detection. *Am J Public Health* 1986;76:512–514.
18. Hanlon JT, Caiola SM, Muhlbaier LH, Dennis BH, Edelman DA, Dingfelder JR. An evaluation of the sensitivity of five home pregnancy tests to known concentrations of human chorionic gonadotropin. *Am J Obstet Gynecol* 1982;144:778–782.
19. Hicks JM, Iosephsohn M. Reliability of home pregnancy test kits in the hands of laypersons. *N Engl J Med* 1989;320:320–321.
20. van Weemen BK. Reliability of home-use pregnancy tests. *Clin Chem* 1993;39:2031–2032.
21. Daviaud J, Fournet D, Ballongue C, *et al*. Reliability and feasibility of pregnancy home-use tests: laboratory validation and diagnostic evaluation by 638 volunteers. *Clin Chem* 1993;39:53–59.
22. Bastian LA, Nanda K, Hasselblad V, Simel D. Diagnostic efficiency of home pregnancy test kits. *Arch Fam Med* 1998;7:465–469.