Clinical Chemistry 60:1
18–21 (2014)

Reflection

The Long Gestation of the Modern Home Pregnancy Test

Glenn D. Braunstein[1*]

The ability of women to confirm their pregnancies in the privacy of their own homes was described as a "private little revolution" 35 years ago, when the early version of the modern home pregnancy test, e.p.t (Warner Chilcott), made its debut in the US *(1)*. Indeed, pregnancy tests are among the most widely used home diagnostic tests, having accounted for $228 million in sales in 2012 (http://www.ibisworld.com/industry/pregnancy-test-kit-manufacturing.html?partnerid=prweb). The development of these tests, which are based on the detection of human chorionic gonadotropin (hCG)[2] in the urine, came 50 years after the discovery of hCG by Aschheim and Zondek *(2)* and after several centuries of descriptions of many other pregnancy tests.

The first pregnancy test to be recorded was written around 1350 BC in the Berlin medical papyrus: "Barley [and] wheat, let the woman water [them] with her urine every day with dates [and] the sand, in two bags. If they [both] grow, she will bear. If the barley grows, it means a male child. If the wheat grows, it means a female child. If both do not grow, she will not bear at all" *(3)*. It is likely that the estrogens (and possibly other growth factors) present in pregnancy urine accounts for the growth stimulation of plants in this and other related plant-based tests described in papyri and later texts. During the Middle Ages, physicians described granules, turbidity, and color changes in urine obtained from pregnant women *(3)*. The physicians who diagnosed pregnancy and a host of other medical conditions through examination of the urine were known as "piss prophets," and they probably arrived at their diagnoses more from being excellent observers of the patient rather than of her urine (Fig. 1).

Although others had described a gonadotropic substance present in the placenta, it was Aschheim and Zondek who discovered hCG in the urine in their demonstration of ovarian stimulation of immature female mice injected with the urine of pregnant women *(2, 3)*. This first bioassay had an analytical sensitivity of 3000–5000 IU/L of urine and took 5 days for a result. Subsequently, numerous other bioassays were described. The end points of these assays were the direct effects of hCG, such as ovulation, ovarian hyperemia, ovarian weight increase, ovarian ascorbic acid depletion, or sperm expulsion (in toads), or the indirect effects of gonadal stimulation, including uterine or ventral prostate increase *(3)*. These assays had analytical sensitivities that ranged from 100 IU/L to 18 000 IU/L and required multiple animals for the necessary precision and 2–9 days for a result *(3)*.

The first immunoassay, a hemagglutination inhibition test, was described in a PhD thesis by Strausser in 1958, but it was never published in the peer-reviewed literature *(4)*. The first published report appeared in 1960, when Wide and Gemzell used a passive hemagglutination inhibition technique to measure hCG *(4)*. In this assay, hCG was adsorbed to the surface of tannic acid–treated sheep erythrocytes and then mixed with a patient's urine and anti-hCG antibodies. In the absence of hCG, the antibodies bound to the erythrocyte-coated hCG and caused agglutination of the red cells. If hCG was present in the urine, it bound to the antibodies and not to the erythrocytes, and hemagglutination was inhibited. This test was relatively inexpensive and rapid (results in 2 h), could measure 200 IU of hCG per liter of urine, and was marketed in 1962 as Pregnosticon (Organon). Similar principles were used to develop the latex agglutination inhibition tube and slide pregnancy tests. Some of the results were available in 2 min and were sensitive to about 1000 IU/L of urine *(3)*. These tests were widely used to diagnose pregnancy in providers' offices during the 1970s and 1980s.

In 1966, Midgley described the first RIA for hCG and its close relative, human luteinizing hormone (hLH) *(5)*. This assay required 3 days to run and had an analytical sensitivity of 175 IU/L. A radioreceptor assay that used hLH/hCG receptors from bovine corpora lutea to capture hCG instead of anti-hCG antibodies was developed by Saxena and colleagues *(6)*. It required 1 h to perform and was sensitive to 5 IU/L. Neither of these assays could discriminate between hCG and hLH; therefore, to measure hCG specifically in pregnancy serum or urine samples required setting the detection limit at a value above the highest physiological hLH concentration that could be seen during the midcycle spike in ovulating women or above the con-

[1] Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.
* Address correspondence to the author at: Department of Medicine, Rm. B118, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048. Fax 310-423-0437; e-mail braunstein@cshs.org.
Received April 22, 2013; accepted July 18, 2013.
Previously published online at DOI: 10.1373/clinchem.2013.202655
[2] Nonstandard abbreviations: hCG, human chorionic gonadotropin; hLH, human luteinizing hormone.

Fig. 1. ***The Doctor's Visit.*** **1667. Oil on panel (44.5 × 31.1 cm) by Dutch artist Frans van Mieris the Elder.**

As a maid attempts to revive a young woman who has fainted, a "piss prophet" examines a vial of her urine. A girl in the background holds a burning ribbon, a symbol of pregnancy. ©Reproduced with permission from The J. Paul Getty Museum, Los Angeles.

centrations found in postmenopausal women, generally around 150–200 IU/L.

The analytical-specificity issue was overcome when the structure of hCG was elucidated in a series of elegant biochemical studies carried out in the laboratories of Drs. Robert E. Canfield at the Columbia University College of Physicians and Surgeons and Om P. Bahl at the State University in Buffalo. They demonstrated that hCG is composed of 2 biologically inactive subunits, α and β, that are noncovalently linked to form the holo (intact) hCG molecule. The α subunits of the 4 glycoprotein hormones, hCG, hLH, follicle-stimulating hormone, and thyroid-stimulating hormone are virtually identical, whereas the β subunits differ. These differences are responsible for the unique biological and immunologic activities of the intact hormones. The β subunits of hCG and hLH share about 80% homology in their first 115 amino acid residues, but the β subunit of hCG has an additional 24 residues at the carboxyterminal end. This difference, along with a greater carbohydrate content, accounts for the major differences between hCG and hLH *(7)*. Thus, it is not surprising that hCG and hLH both react with the hLH/hCG receptors on target tissues or that when the intact hormone is injected into rabbits or other species, the antibodies produced recognize hCG and hLH equally well. Until 1971, the only way to measure low hCG concentrations specifically in the presence of hLH was to separate them by column chromatography and then measure hCG in the effluent tubes by RIA, because hCG eluted earlier than hLH.

In 1970, Dr. Judith Vaitukaitis and I began to work with Dr. Griff Ross at the NIH, initially in the Endocrine Research Branch of the National Cancer Institute and then the Reproductive Research Branch of the National Institute of Child Health and Human Development. Bob Canfield had just provided Griff with purified hCG and its subunits, and we were going to characterize the proteins immunochemically. Judy collaborated with Dr. John Robbins, who had developed a technique for immunizing rabbits with small quantities of immunogen. She used 10 and 50 μg of hCG, and the first animal she immunized with the 50-μg dose produced a high-titer antiserum that was relatively specific for hCG *(8)*. We used this antibody, named SB6 after the rabbit that produced it (the sixth rabbit receiving the β subunit), to develop the original β-hCG RIA. This assay was capable of measuring as little as 5 IU/L of hCG in serum in the presence of high physiological amounts of hLH *(9)*. It was subsequently shown that new epitopes (including the carboxyterminal portion) are revealed when the hCG subunits are separated and that antibodies reacting both with hCG β subunits and with hCG are generated when the hCG β subunit is used as an immunogen. These antibodies are more specific for hCG than for hLH, as are some antibodies that recognize conformation-dependent epitopes on the heterodimeric hCG molecule *(10)*.

With this β-hCG RIA, we were able to detect hCG (or its β subunit) as early as 7.5 days after fertilization, and we used the assay to establish the reference interval for serum hCG concentrations throughout pregnancy, to describe the phenomenon of subclinical spontaneous abortion (in which the women conceived but miscarried so early that they did not know they had been pregnant), and to establish hCG values in a variety of early-pregnancy disorders *(11–14)*. We also were able to detect low residual hCG concentrations in patients with gestational trophoblastic disease, who had previously been determined to be cured with the aid of conventional hCG bioassays of 24-h urine concentrations. In addition, measurement of hCG (or its β subunit) in serum was used for patients with testicular germ cell tumors and ectopic nontrophoblastic hCG- or β-hCG–

producing neoplasms *(15, 16)*. Although the initial β-hCG RIA was developed to measure hCG in serum, the assay was easily modified to eliminate matrix effects so it could be used for measuring hCG in urine *(17)* and a variety of healthy human tissues *(18)*. Many manufacturers produced β-hCG RIAs for use in commercial laboratories, and over the years, these assays have evolved from hand-pipetted samples and reagents to automated platforms, replaced radioactive labeling with enzyme labeling, improved in analytical sensitivity and specificity, and led to the development of assays that can measure very specific moieties and isoforms of hCG, including intact hCG, β-hCG, nicked hCG, nicked β-hCG, hCG β-core fragment, and hyperglycosylated hCG *(7)*.

As noted above, the first over-the-counter pregnancy test for home use in the US, e.p.t® (standing for "early pregnancy test"), was produced by Warner Chilcott. It was a hemagglutination inhibition test that required 2 h to perform. Home pregnancy tests were actually available earlier in the 1970s in Europe and Canada. These early kits had relatively poor analytical sensitivity and required 500 IU/L to 2000 IU/L of hCG to give a positive reaction and to avoid false-positive results due to cross-reaction with physiological concentrations of hLH. In addition, these tests were fraught with technical difficulties, such as interference by proteinuria, hematuria, medications, vibration (tube tests), and user error. These issues were overcome with the development of several technologies, including murine monoclonal antibodies generated against specific epitopes of the hCG molecule and its subunits; new materials and methodologies for labeling antibodies with gold, silver, carbon, selenium, or latex particles (in addition to enzymes); and advances in solid-phase matrix chemistry. Modern home pregnancy tests are 2-site immunochromatographic assays and often contain a labeled anti–β-hCG antibody, antiimmunoglobulin antibodies to eliminate nonspecific interference, and anti-LH "scavenger" antibodies in the mobile phase. When urine is placed on the membrane, these mobile-phase antibodies move down the nylon or nitrocellulose membrane by capillary action. When they reach the capture antibody, which is often directed against the α subunit or another epitope on the β subunit, the hCG-labeled/anti–β-hCG complex is captured to produce a positive reaction. These tests also have an internal control, which usually consists of an antimouse immunoglobulin that reacts with the labeled mouse anti–β-hCG antibody to indicate that the urine volume was sufficient to expose the test zone to the sample.

More than 60 brands of home pregnancy tests are available in the US market, although many of the brands are produced by the same manufacturers. Their analytical sensitivities range from 6.3 IU/L to 50 IU/L of urine, with most falling between 20 and 25 IU/L (http://www.fertilityplus.com/faq/hpt.html). Even with these very analytically sensitive and specific tests, issues with false-negative results do occur when women test their urine too early, although advertisements for some tests state that a positive test result can be obtained several days before a missed menses. The reasons for a false-negative result include the observations that up to 10% of women may not even have implanted on the day of their expected menses, the amount of hCG secreted by the trophoblast during early pregnancy can vary by 10-fold, the monoclonal antibodies used in the test may not recognize the major forms of hCG present in the urine during early pregnancy, high concentrations of the β-core fragment of hCG can produce interference in some of the tests, and, rarely, the high-dose hook effect can occur *(13, 19–21)*.

Getting to where pregnancy can be diagnosed accurately at home within 2 to 3 weeks after conception has been a remarkable journey. It certainly has helped to empower women to maintain control over a portion of their reproductive lives, and the fact that about one-third of reproductive-age women have used these tests (http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/TipsandArticlesonDeviceSafety/ucm109396.htm#) attests to the popularity of these tests. I am thankful to have been lucky enough to be at the right place, at the right time, and with the right colleagues to make a contribution to that journey.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: G.D. Braunstein, LabCorp and Abbott Diagnostics.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.

References

1. Leavitt SA. "A private little revolution": the home pregnancy test in American culture. Bull Hist Med 2006;80:317–45.
2. Aschheim S, Zondek B. Hypophysenvorderlappenhormon und Ovarialhormon im Harn von Schwangeren. Klin Wochenschr 1927;6:1322.
3. Burstein J, Braunstein GD. Urine pregnancy tests from antiquity to the present. Early Pregnancy 1995;1:288–96.
4. Wide L. Inventions leading to the development of the diagnostic test kid industry—from the modern pregnancy test to the sandwich assays. Upsala J Med Sci 2005;110:193–216.
5. Midgley AR Jr. Radioimmunoassay: a method for human chorionic gonadotropin and human luteinizing hormone. Endocrinology 1966;79:10–8.
6. Saxena B, Hasan SH, Haour F, Schmidt-Gollwitzer M. Radioreceptor assay of human chorionic gonadotropin: detection of early pregnancy. Science 1974;184:793–5.
7. Stenman UH, Tiitinen A, Alfthan H, Valmu L. The classification, functions and clinical use of different isoforms of hCG. Hum Reprod Update 2006; 12:769–84.
8. Vaitukaitis JL. Development of the home pregnancy test. Ann N Y Acad Sci 2004;1038:220–2.
9. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gyencol 1972; 113:751–8.
10. Medeiros SF, Norman RJ. Human choriogonadotrophin protein core and sugar branches heterogeneity: basic and clinical insights. Hum Reprod Update 2009;15:69–95.
11. Braunstein GD, Grodin JM, Vaitukaitis J, Ross G. Secretory rates of human chorionic gonadotropin by normal trophoblast. Am J Obstet Gynecol 1973;115:447–50.
12. Braunstein GD, Rasor J, Adler D, Danzer H, Wade M. Serum human chorionic gonadotropin levels throughout normal pregnancy. Am J Obstet Gynecol 1976;126:678–81.
13. Braunstein GD, Karow WG, Gentry WD, Wade ME. Subclinical spontaneous abortion. Obstet Gynecol 1977;50(1 Suppl):41s–4s.
14. Braunstein GD, Karow WG, Gentry WC, Rasor J, Wade ME. First-trimester chorionic gonadotropin measurements as an aid in the diagnosis of early pregnancy disorders. Am J Obstet Gynecol 1978; 131:25–32.
15. Braunstein GD, Vaitukaitis JL, Carbone PP, Ross GT. Ectopic production of human chorionic gonadotropin by neoplasms. Ann Intern Med 1973;78:39–45.
16. Braunstein GD, McIntire KR, Waldmann TA. Discordance of human chorionic gonadotropin and alpha-fetoprotein in testicular teratocarcinomas. Cancer 1973;31:1065–8.
17. McCready J, Braunstein GD, Helm D, Wade ME. Modification of the choriogonadotropin beta-subunit radioimmunoassay for determination of urinary choriogonadotropin. Clin Chem 1978;24: 1958–61.
18. Braunstein GD, Kamdar V, Rasor J, Swaminathan N, Wade M. Widespread distribution of a chorionic gonadotropin-like substance in normal human tissues. J Clin Endocrinol Metab 1979;49: 917–92.
19. Wilcox AJ, Baird DD, Dunson D, McChesney R, Weingberg CR. Natural limits of pregnancy testing in relation to the expected menstrual period. JAMA 2001;286:1759–61.
20. Butler SA, Khanlian SA, Cole LA. Detection of early pregnancy forms of human chorionic gonadotropin by home pregnancy test devices. Clin Chem 2001;47:2131–6.
21. Gronowski AM, Cervinski M, Stenman UH, Woodworth A, Ashby L, Scott MG. False-negative results in point-of-care qualitative human chorionic gonadotropin (hCG) devices due to excess hCGβ core fragment. Clin Chem 2009;55:1389–94.