
2 Human Chorionic Gonadotropin

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INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein composed of two dissimilar subunits, α - and β -subunit, held together by charge interactions. hCG is produced by trophoblastic cells of the placenta in both pregnancy and gestational trophoblastic diseases. It is a remarkable glycoprotein in that up to 35% of the molecular weight (MW) is from oligosaccharide side chains. hCG is sometimes considered a mucopolysaccharide, like collagen, because of the large carbohydrate component. There is wide variation in hCG structure throughout normal and abnormal pregnancies, and in gestational trophoblastic diseases. In addition to “regular” hCG (hCG with intact subunits and the midtrimester pregnancy-like complement of oligosaccharides), at least six other key variants are present in serum samples: hyperglycosylated hCG, nicked hCG, hCG missing the β -subunit C-terminal peptide, free β -subunit, hyperglycosylated free β -subunit, and nicked free β -subunit as well as multiple combinations of these variants (i.e., nicked hyperglycosylated hCG missing the β -subunit C-terminal peptide). The same seven molecules plus the β -core fragment can be detected in urine samples (1–9). Table 1 and Fig. 1 summarize the structures of these key hCG-related molecules, which vary in size from β -core fragment (MW 9000–10,000) to hyperglycosylated hCG (MW 38,000–42,000). hCG and related molecules may vary widely in charge because of differences in sialic acid content. As shown in Fig. 2, multiple charge isoforms in the range pI 3–7 are found in serum and urine samples, and in the range of pI 3–8 in hyperglycosylated hCG.

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Table 1
Structure of hCG-Related Molecules Detected in Serum and Urine Samples
in Normal and Abnormal Pregnancies and Trophoblastic Diseases (1–9)

<i>Molecule</i>	<i>α-subunit structure</i>	<i>β-subunit structure</i>	<i>Occurrence</i>
Regular hCG (MW 37,000)	92 amino acids, no cleavages mono- and biantennary <i>N</i> -linked oligosaccharides	145 amino acids, no cleavages biantennary ± fucose <i>N</i> -linked, and mostly tri- and tetrasaccharide <i>O</i> -linked oligosaccharides	Principal form of βhCG in serum, 6–40 wk of gestation
Hyperglycosylated hCG (MW 41,000)	92 amino acids, no cleavages mono- and biantennary + fucose <i>N</i> -linked oligosaccharides	145 amino acids, no cleavages bi-, short tri-, and triantennary ± fucose <i>N</i> -linked and hexasaccharide <i>O</i> -linked oligosaccharides	Principal βhCG form produced at 3–5 wk of gestation, and in choriocarcinoma
Nicked hCG ¹ (MW 36,000)	92 amino acids, no cleavages mono- and bi-antennary <i>N</i> -linked oligosaccharides	145 amino acids, cleaved at β47–48, β43–44 or β44–45 biantennary ± fucose <i>N</i> -linked, and mostly tri- and tetrasaccharide <i>O</i> -linked oligosac- charides	Low concentrations in pregnancy, major hCG in weeks following termination or parturition
hCG missing β-subunit C-terminal peptide ¹ cancer (MW 29,000)	92 amino acids, no cleavages mono- (8 sugars) and biantennary <i>N</i> -linked oligosaccharides	Residues 1–92, C-terminal peptide absent biantennary ± fucose <i>N</i> -linked, and mostly tri- and tetra- saccharide <i>O</i> -linked oligosaccharides	Primarily detected in gestational trophoblastic diseases and
Free β-subunit ¹ (MW 22,000)	No α-subunit	145 amino acids, no cleavages biantennary ± fucose <i>N</i> -linked, and mostly tri- and tetrasaccha- ride <i>O</i> -linked oligosaccharides	Dissociation product of nicked hCG. Low levels in serum and urine in pregnancy

continued

Table 1 (continued)

<i>Molecule</i>	<i>α-subunit structure</i>	<i>β-subunit structure</i>	<i>Occurrence</i>
Hyperglycosylated free β-subunit ¹ (MW 26,000)	No α-subunit	145 amino acids, no cleavages bi-, short tri- and triantennary ± fucose <i>N</i> -linked and hexasaccharide <i>O</i> -linked oligosaccharides	Significant production at 3–5 wk of gestation, and in choriocarcinoma
Nicked free β-subunit ¹ (MW 22,000)	No α-subunit	145 amino acids, cleaved at β47–48, β43–44 or β44–45 biantennary ± fucose <i>N</i> -linked, and mostly tri- and tetrasaccharide <i>O</i> -linked oligosaccharides	Most free β-subunit is nicked in serum or is dissociated from nicked hCG
Urine β-core fragment (MW 10,000)	No α-subunit	Two peptides β-subunit residues 6–40 linked to 55–92 degraded biantennary <i>N</i> -linked and no <i>O</i> -linked side chains	Cleared from circulation rapidly. Principal of β-hCG in urine from 7–40 wk of gestation

¹Variations of these hCG variants are present in serum and urine, such as hyperglycosylated nicked hCG, nicked hCG missing β-subunit C-terminal peptide, nicked hyperglycosylated free β-subunit, nicked free β-subunit missing the C-terminal peptide, and others.

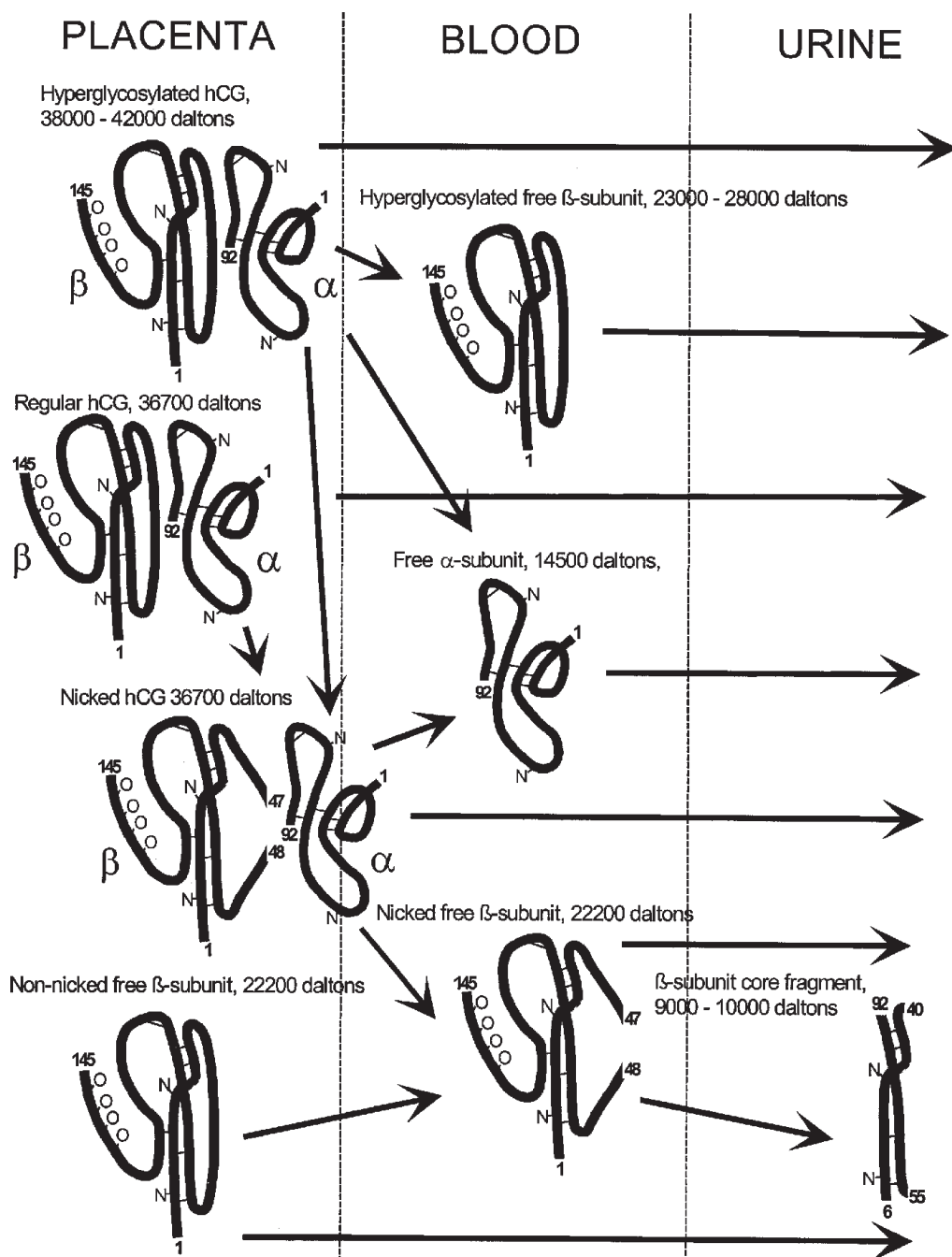


Fig. 1. Two-dimensional representation of structure and molecular weights of hCG forms produced by the placenta and present in serum and urine samples. Dark black lines represent peptide with numbers indicating N- and C-terminal residues. The letters *N* and *O* indicate sites of N- and O-linked oligosaccharides, and the thin lines indicate sites of disulfide linkages. Representations are based on the β core fragment sequence as shown by Birken et al. (40), amino acid sequences as reported by Morgan et al. (41), disulfide bonds as shown by Lapthorn et al. (42), and the nicking sites as indicated by Elliott et al. (7).

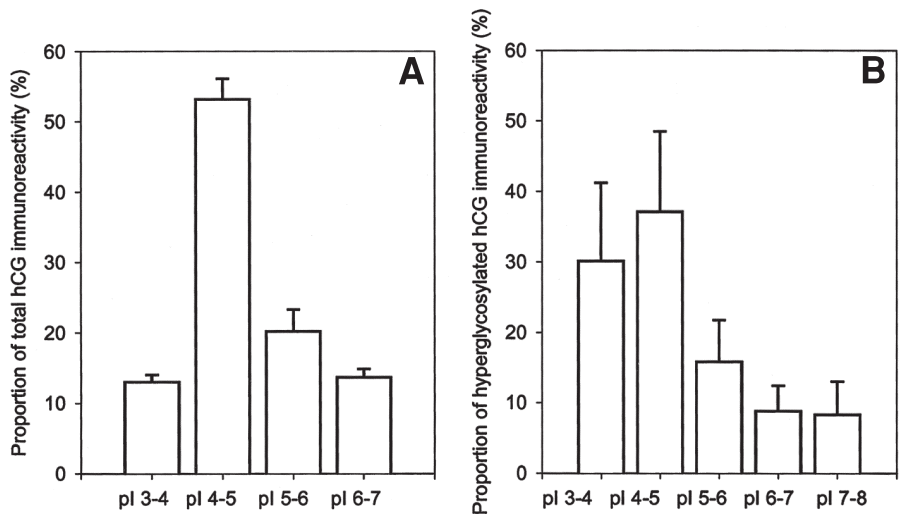


Fig. 2. Charge isoforms of hCG and hyperglycosylated hCG. Pregnancy urine samples were separated into 20 fractions by preparative isoelectric focusing using a Bio-Rad. Panel A shows total hCG isoforms in six samples (Rotofor DPC Immulite hCG assay); Panel B shows hyperglycosylated hCG isoforms in eight further samples (Nichols Advantage hyperglycosylated hCG [ITA] test). All results are distribution of isoforms (% $\pm$ standard error).

hCG PRODUCTION DURING PREGNANCY

hCG production does not begin until the developing blastocyst implants into the uterus. Circulating hCG can first be detected (following implantation of the fertilized egg) in serum and urine, with an ultrasensitive hCG test (detection limit 0.13 mIU/mL) as early as day 21 of the menstrual cycle (approx 7 d after fertilization) (10). Total hCG concentrations in serum and urine samples increase exponentially, doubling approximately every 40–48 h, and reach a peak between 8 and 12 wk of pregnancy (Fig. 3) (10a,10b). Total hCG production decreases between the 10th and 20th wk of pregnancy to between one-fifth and one-twentieth of peak hCG concentrations and then plateaus close to this concentration until term. Pregnancy hCG concentrations vary widely among individuals, probably more than any other hormone measurement. This is complicated further by interassay variation in quantitative serum hCG tests (*see* “Interassay Variation”). Table 2 illustrates the range of serum hCG results we have observed with the DPC Immulite (Diagnostic Products Corp., Los Angeles, CA) test in 300 serum samples. Table 2 is limited to hCG measurements over 4-wk periods and to ranges of concentrations; therefore, it is used only to illustrate the wide range of hCG results during pregnancy and not as a reference interval for different hCG tests or for patient care. As shown in Table 2, for any 4-wk period of pregnancy there is anywhere from a 10- to 200,000-fold variation in interindividual serum hCG concentrations.

Table 3 illustrates median concentrations and ranges for urine total β -hCG concentrations throughout the course of pregnancy, as determined using the DPC Immulite test. In our experience, first morning urine total β -hCG concentrations (including hyperglycosylated hCG and β -core fragment) are approximately one-half of serum total β -hCG concentrations. As shown in Table 3, for any one period of pregnancy there is approximately a 10- to 4000-fold variation in interindividual urine hCG concentrations.

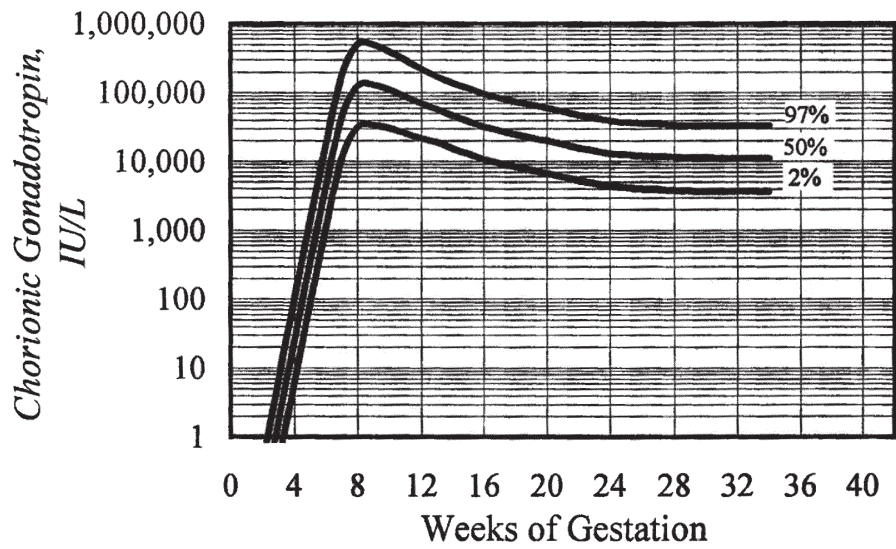


Fig. 3. Concentration of chorionic gonadotropin in maternal serum as a function of gestational age. Lines represent the 2nd, 50th, and 97th percentiles. The maternal serum values from 14 to 25 wk are medians calculated from 24,229 pregnancies from testing performed at ARUP Laboratories, Inc., from January to October 1997. Evaluating health and maturation of the unborn: the role of the clinical laboratory. Clin Chem, 1992;38:1523–1529. Reprinted with permission from W.B. Saunders Co. Aswood, ER. Clinical chemistry of pregnancy. In Burtis CA, Ashwood, ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders, 1998, pp. 1736–1775. (Redrawn from ref. 10a: Reprinted with permission from W.B. Saunders [10b].)

Table 2
Range of Total β -hCG Concentrations in 300 Serum Samples Collected
During the Course of Pregnancy, as Determined
in the DPC Immulite hCG Assay

Gestational age (wk)	Total β -hCG range (mIU/mL)
4–7	0–233,200
8–11	11,440–465,300
12–15	14,300–510,400
16–19	6490–337,700
20–23	550–72,930
24–27	9240–107,470
28–31	12,760–152,900
32–35	12,980–128,480
35–39	5390–114,730

Table 3
Median and Range of Total β -hCG and Hyperglycosylated hCG Concentrations in Urine

<i>Gestational age (wk)^a</i>	<i>Number of samples</i>	<i>Total β-hCG^b mIU/mL</i>	<i>Range</i>	<i>H-hCG^c mIU/mL</i>	<i>Range</i>	<i>Proportion H-hCG^d (% of hCG)</i>	<i>Range</i>
4	63	454	(23–4653)	282	(5–7513)	68%	(7–100%)
5	45	1615	(114–45,800)	779	(35–17,479)	50%	(10–100%)
6	14	2678	(187–20,439)	483	(37–5931)	25%	(7.9–53%)
7	5	16,649	(1608–59,277)	1871	(333–14,055)	13%	(2–24%)
11	20	24,550	(5534–100,222)	1997	(180–13,152)	10%	(0.8–55%)
15	92	8371	(2298–43,932)	290	(28–2906)	3%	(0.2–48%)
19	12	4452	(2278–24,228)	125	(25–797)	2.5%	(0.4–17%)
23	13	3702	(1129–20,725)	32	(11–411)	1.1%	(0.1–12%)
27	12	4600	(840–10,266)	41	(17–473)	2.0%	(0.2–7.5%)
31	7	4449	(1651–39,549)	55	(11–446)	2.0%	(0.1–8.7%)
35	16	5430	(1399–62,500)	78	(17–587)	1.5%	(0.3–14%)
39	16	2379	(276–13,321)	34	(6–260)	1.9%	(0.5–7.4%)

^aWeeks since last menstrual period.

^bDetermined using DPC Immulite.

^cDetermined using the Nichols Advantage, Invasive Trophoblastic Antigen (ITA) assay.

^dThe proportion of hyperglycosylated hCG was determined as the concentration of hyperglycosylated hCG divided by the total β -hCG result.

While regular hCG is produced by highly differentiated multinucleated syncytiotrophoblast cells during pregnancy, hyperglycosylated hCG, also called invasive trophoblast antigen (ITA), is produced by the poorly differentiated, invasive cytotrophoblast cells (2,11). Invasive cytotrophoblasts are the cells of implantation and the primitive cells of early pregnancy. Therefore, as illustrated in Table 3, hyperglycosylated hCG is responsible for most of the total hCG immunoreactivity in the third, fourth and fifth weeks of pregnancy (1,3). This is true for both serum and urine samples. This early period of pregnancy is the most frequent time for hCG testing, yet, as described in “Interassay Variation,” assays vary greatly in their ability to detect hyperglycosylated hCG. Underestimation and overestimation of this critical pregnancy marker occurs frequently (3,4).

PRINCIPALS OF hCG TESTS

More than 40 quantitative serum hCG assays, approx 30 qualitative urine/serum point-of-care tests, and 25 qualitative urine home pregnancy tests are available in the United States for detecting pregnancy (4,12). These assays are all “sandwich”-type immunoassays that utilize capture and detection antibodies that recognize different sites on hCG and hCG-related molecules.

Quantitative hCG Tests

In quantitative serum tests, a capture antibody directed against one site on hCG is immobilized on or linked to a tube, vial, well, or bead. Patient serum is added. The antibody binds and immobilizes the hCG in the serum sample (hCG + immobilized antibody). A second (“detection” or “tracer”) antibody directed against a distant site on hCG is linked to a tracer molecule (such as a radioisotope or an enzyme). This forms an immobilized “sandwich” complex (tracer-antibody + hCG + immobilized antibody). In most commercial hCG assays, both antibodies are directed against different portions of the β chain. Therefore, the assays measure total β -hCG (i.e., free β and intact $\alpha\beta$ molecules). After washing, the amount of tracer is measured (in the case of enzyme assays, incubation with substrate is required). The amount of signal is directly proportional to the amount of hCG in the sample. Quantitative hCG tests are either one step or two step. In a one-step assay, the serum is incubated with both immobilized and tracer antibodies simultaneously. In a two-step assay, the serum is first incubated with the immobilized antibody and then washed before the addition of the tracer antibody. The one-step protocol is by far the most common protocol used. It is the fastest and the least complicated. The slower two-step protocol, however, avoids problems with hook effect and limits false positive hCG results caused by heterophilic antibodies. Both of these issues are discussed later in this chapter under “Limitations of hCG Tests.”

Qualitative hCG Tests

Qualitative hCG assays, whether home pregnancy tests or point-of-care hCG tests, work on a very similar principal to the quantitative assays. Urine is placed on a single-use assay cassette. The urine is absorbed through changing densities of nitrocellulose. This concentrates the antigens into a narrow band. The urine mixes with a colored-dye-labeled anti-hCG antibody (“tracer” antibody) stored in a sponge at the proximal end of the device. The combination of urine and colored-dye-labeled anti-hCG antibody slowly moves distally through the absorbent nitrocellulose. A complex is formed (hCG + anti-

body dye). The complex reaches a stationary band of immobilized secondary “capture” antibody in the nitrocellulose matrix. This antibody is directed against an alternative binding site on the hCG molecule. These devices are designed to recognize intact hCG, so one antibody is against the α chain, the other against the β chain. An antibody dye + hCG + immobilized antibody complex is formed that generates a colored line at the site of the “capture” hCG antibody. This is referred to as the “test band.” The appearance of a colored line indicates a positive test result (generally at concentrations >20 mIU/mL). Remaining antibody dye migrates further through the device to a second stationary band referred to as the “control band.” What is immobilized on the control band varies from test to test. The optimal control band is coated with all or part of the hCG molecule. Excess antibody dye will bind the immobilized hCG, indicating that there was both enough volume to carry the sample across the nitrocellulose and that the antibody dye is working correctly. Unfortunately, not all devices contain this type of control. Some devices have a control band coated with anti dye antibody. This type of control merely indicates that there was enough volume to carry the antibody dye to that distal point.

Antibody Selection

All of these tests, whether quantitative or qualitative, employ at least two different antibodies, whether monoclonal or polyclonal. These are generated against a combination of any of seven established epitopes on hCG. These are the outer section of the β -subunit C-terminal peptide (CTP outer: residues 123–145 plus other hCG three-dimensional [3D] folded structures), the inner section of the CTP (CTP inner: residues 93–122 plus hCG 3D folded structures), at least two separate sites in the core 3D folded structure of the β -subunit (core β -subunit sites 1 and 2), the interface of the α - and β -subunits (anti-hCG, 3D structures on both subunits), and a specific site in the core 3D folded structure of α -subunit (core α -subunit site) (Table 4). In addition, there are specific antibody binding sites for hyperglycosylated hCG only, free β -subunit only, free β -subunit plus β -core fragment β -core fragment only, nicked hCG only, and free α -subunit only. Examining the different antibody combinations used in today’s hCG tests shows that manufacturers are effectively using almost all combinations of these seven epitopes on hCG and the seven epitopes plus free subunit antibodies for their tracer and immobilized antibody (12). As a result, there is very large number of different types of hCG assays, whether quantitative or qualitative. These vary widely in their detection of the eight key variants of hCG. Whereas some tests in each category measure only regular hCG, others detect all eight key variants from hyperglycosylated hCG to β -core fragment (Table 4). This is a major source of interassay variation in the results of quantitative serum tests and in the sensitivities and utilities of qualitative tests (*see* “Interassay Variation”).

All tests use at least one antibody directed against one of the sites on the β -subunit of hCG to differentiate hCG from luteinizing hormone, thyroid-stimulating hormone, and follicle-stimulating hormone. Some assays use a secondary antibody against the α chain, others utilize two β chain antibodies. Thus, the term β hCG test is commonly used for an hCG test. For the purpose of this chapter, an hCG test and a β hCG test are the same.

hCG STANDARDS

All hCG tests, whether quantitative or qualitative, are standardized with World Health Organization (WHO) international hCG standards. WHO standards are calibrated in

Table 4
Antigenic Determinants on hCG, Free β , Free α , and β -Core and Derivatives

		Antigen specificity									
Antigenic determinant	Description	Free α -subunit									
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+ indicates recognized by all polyclonal and monoclonal antibody against this antigenic determinant.

± indicates recognized poorly or recognized by some polyclonal and monoclonal antibodies to this determinant.

international units (IU). The use of international units dates back to the rabbit biological test of the 1940s (12). The first WHO international standard for hCG was the First International Standard (1st IS), released in 1938. This was followed by the 2nd IS, released in 1964. These were both crude hCG preparations, made from pregnancy urine extracts, and contained a mixture of biologically active hCG, free subunits, and other substances. In 1978, the 2nd IS was replaced by the First International Reference Preparation (1st IRP, also known as hCG preparation 75/735), prepared from a highly purified hCG preparation (CR119) from the National Institutes of Health (13). The 1st IRP contained a defined mass concentration of hCG but the designation of international units was kept for compatibility with the 2nd IS ($1 \mu\text{g hCG} = 9.3 \text{ IU}$; 14). On a weight basis, the 2nd IS has approximately one-half the biological activity of the 1st IRP. The CR119 preparation was later used to make the 3rd IS in 1986 (hCG preparation 75/537) and, more recently, the 4th IS (hCG preparation 75/589), released in 1999. Currently, all tests are calibrated with either the 3rd IS or the 4th IS.

The CR119 series of standards was purified from crude pregnancy urine preparation that was collected and stored at ambient temperature, which allowed for aberrant cleavage or nicking of hCG. Research shows that 9% of the 1st IRP, 3rd IS and 4th IS hCG molecules are nicked or damaged in the region of β -subunit residues 43–48 (15). This presented a problem with assays that failed to recognize or poorly detected nicked hCG molecules (12,16). A new hCG standard was released in 2003, isolated from a new commercial crude pregnancy urine extract. This will be referred to as the 1st reference reagent (1st RR, hCG preparation 99/688). To improve assay recognition and reduce interassay variation, this standard has been further purified to remove enzyme impurities and the small proportion of nicked hCG molecules that were present in previous standard hCG preparations (16).

As discussed under “Interassay Variation,” the availability of WHO standards to normalize hCG test results and cutoff values is important. All manufacturers purchase WHO standards for calibrating tests. The quantities, however, are very limited and not sufficient to provide calibrators and quality control material for qualitative products. As such, almost all manufacturers purchase crude or partially purified urine-derived hCG either from Scripps Laboratories (La Jolla, CA), Organon (West Orange, NJ), or other suppliers. These preparations are calibrated with the WHO standard and subsequently used as calibrators and/or standards. This is problematic as these preparations are not nearly as well characterized as the WHO standards and may contain a mixture of hCG, nicked hCG, free β -subunit, and β -core fragment. As a result, they are recognized differently by different quantitative hCG tests and may calibrate qualitative pregnancy tests differently. This is likely a major cause of interassay variation and may explain why two serum hCG tests can give different results and why one qualitative test may be positive and another negative with the same urine sample (17).

INTERASSAY VARIATION

Interassay variation between different quantitative serum tests and between different qualitative urine tests can cause confusion and misdiagnosis of pregnancy. Most clinical laboratories participate in external proficiency testing through the College of American Pathologists (CAP). CAP surveys have been the biggest indicator of interassay variation. The five most recent CAP surveys (each with a single hCG preparation) of 2307 to 2324

testing laboratories showed 1.50- to 1.59-fold variations from the mean in results for the 16 most commonly used hCG assays in the United States (18). In 1990, Bock wrote an article titled “hCG Assays: A Plea for Uniformity,” strongly criticizing the wide variation in CAP survey hCG results and demanding that manufacturers resolve these differences (19). Clearly, the plea was not effective and the problem, although slightly less pronounced, still exists today. Because of the differences between assays, individual assay-specific reference intervals must be established and should not be interchanged. This can be a source of confusion when hCG is measured at different medical centers.

Figure 4a illustrates the interassay variation in the 10 most commonly used hCG tests from the most recent CAP survey report (18). We recently requested that 11 laboratories quantitate the 1st RR, the newest, purest, and supposedly the most homogenous WHO hCG standard available (16), using the same 10 hCG tests (each calibrated against the current 3rd IS). As shown in Fig. 3, Panel B, wide variation in results was recorded between assays (1.6-fold variation). Interestingly, the tests giving the highest and lowest results with the 1st RR standard [the Bayer Centaur (Bayer, Medfield, MA) and Roche Elecsys (Roche Diagnostics, Indianapolis, IN) tests, respectively] are completely different than those giving the highest results and lowest results with CAP proficiency test material [the DPC Immulite and Beckman Access-2 (Beckman Coulter, Fullerton, CA), respectively]. Similarly, when the same 10 assays were calibrated directly with the 1st RR, 1.6-fold variation remained (data not shown), with the Dade Dimension RXL (Dade Behring, Newark, DE) yielding the highest and the Bayer Centaur the lowest result (17). All of these observations indicate the complexity of interassay variation.

We attribute much of the interassay variation to two main factors: (1) differences in assay specificity, and (2) standardization material. Each will be discussed in more detail below.

Assay Specificity

One of the causes of interassay variation between hCG measurements is the use of antibodies that recognize different hCG molecules and degradation products (Table 4). The manufacturer's choice of antibodies will significantly affect results because antibody specificity will determine the number of different hCG molecules the assay will be able to detect. For instance, the reason the DPC Immulite hCG test consistently gives the highest result with the CAP proficiency test material, and not with the pure 1st RR, is that it is the only commercial hCG test that detects β -core fragment (Fig. 5). Whereas the 1st RR is pure and free of such contaminants, the CAP hCG proficiency test material is derived from a crude urine preparation (CAP technical service; Northfield, IL; personal communication), which likely contains significant β -core fragment, recognized only by the DPC Immulite hCG test. In addition, serum hCG assays differ in their ability to recognize hyperglycosylated hCG (Fig. 5). Quantification of a purified hyperglycosylated hCG standard ranged from 468 IU/L (Dade Dimension RXL) to 1544 IU/L (Roche Elecsys) (9,12,17). This is a 3.3-fold variation. Similarly, serum hCG assays differ in their ability to recognize hCG free β -subunit (2.2-fold variation) and nicked hCG (1.5-fold variation). For a comprehensive list of commercially available hCG immunoassays and their specificity, see Cole (12).

Significant interassay variation is also observed with home pregnancy tests and urine point of care tests. These tests are all approved for use with urine, yet in a recent study, we found that only 2 of 14 home pregnancy tests and a similar low proportion of urine

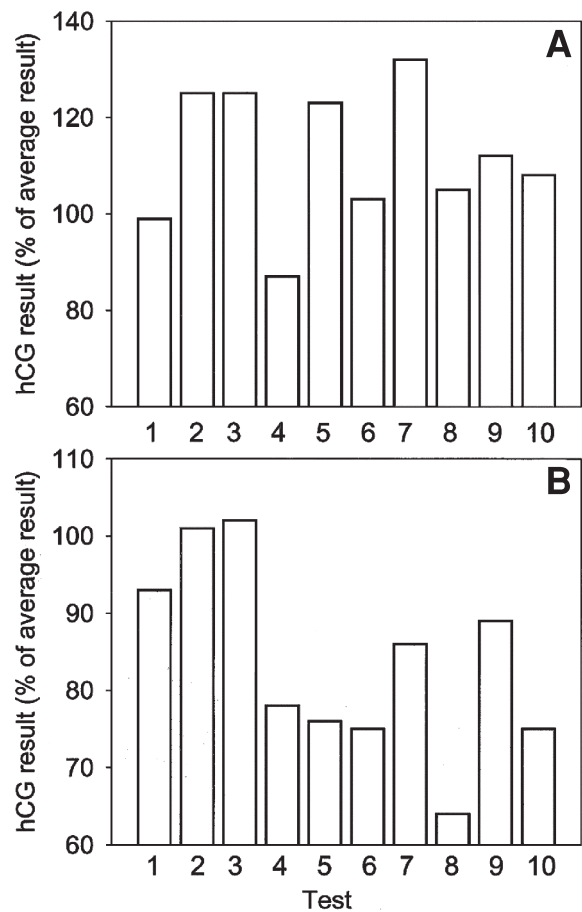


Fig. 4. Interassay variation of hCG tests. Interassay variation of 10 tests was investigated, the Abbott AxSym (test 1), Bayer ACS-180 (test 2), Bayer Centaur (test 3), Beckman Access-2 (test 4), Dade Dimension (test 5), Dade Stratus (test 6), DPC Immulite (test 7), Roche Elecsys (test 8), Tosoh A1A600 (test 9), and Vitros Eci (test 10). Panel A shows interassay variation as reported as part of ongoing CAP interlaboratory comparisons, involving 2307 laboratories (18). Panel B shows a blind 11-laboratory comparison with the same 10 assays and the new 1st RR hCG standard.

point-of-care devices detect β -core fragment, the principal immunoreactive subunit in pregnancy urine throughout gestation (3). These tests are intended for use in early pregnancy detection. However, in a study of 14 home pregnancy test devices, 3 (Rite Aid, Long's, and Inverness Medical One-Step) had poor detection or completely failed to detect hyperglycosylated hCG, the principal hCG molecule produced in the first weeks of gestation. In a more recent study, three other products (Sav-Osco, Target, and CVS one-step tests) were also shown to either not recognize or poorly recognize hyperglycosylated hCG (Cole L, Khanlian S, Sutton J, and Davies S, unpublished data). It was also observed that two products, Answer and Answer Quick and Simple, preferentially detected hyperglycosylated hCG.

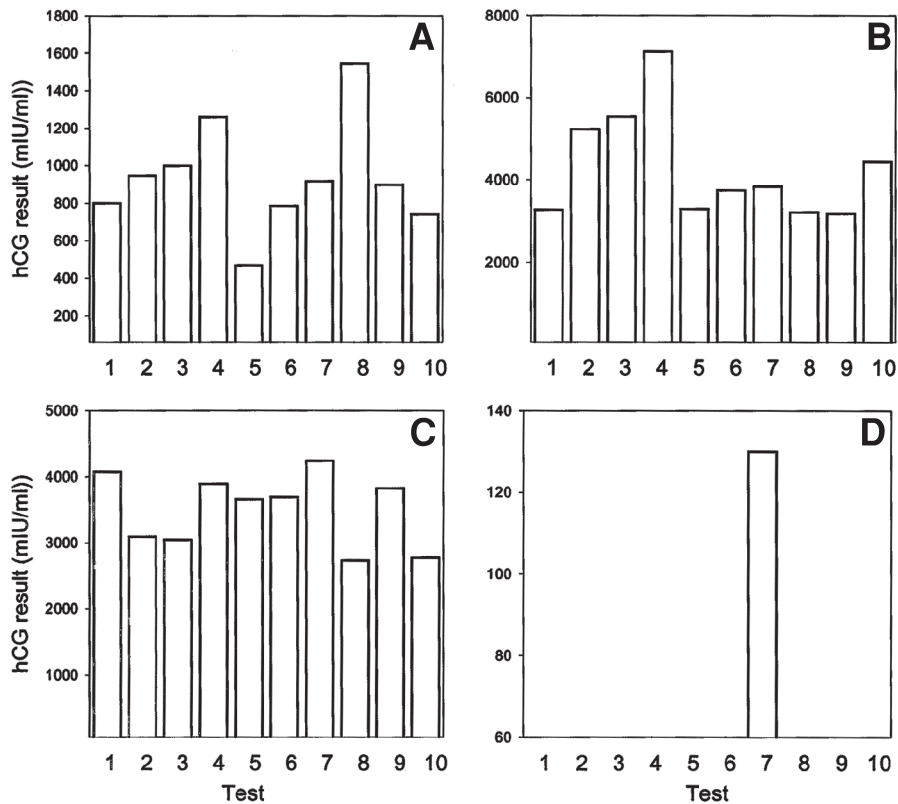


Fig. 5. Interassay variation of hCG tests. Interassay variation of 10 tests was investigated, the Abbott AxSym (test 1), Bayer ACS-180 (test 2), Bayer Centaur (test 3), Beckman Access-2 (test 4), Dade Dimension (test 5), Dade Stratus (test 6), DPC Immulite (test 7), Roche Elecsys (test 8), Tosoh A1A600 (test 9), and Vitros Eci (test 10). All were determined blindly in 11 laboratories (17). Panel A is pure hyperglycosylated hCG, calibrated by mass as 100 µg/L. Panel B is pure free β-subunit (250 µg/L). Panel C is pure nicked hCG, nicked only at β47–48 (7) (400 µg/L). Panel D is pure β-core fragment (50 µg/L).

Standardization Material

The second factor that impacts interassay variation is the supply of pure WHO standards (20). As stated earlier, the quantities of WHO standards are limited and are not sufficient for the provision of calibrators for all laboratories or standards for quality controls. As such, manufacturers purchase crude or partially purified urine-derived hCG and calibrate these with the WHO standard for use as calibrators and quality control material. This can greatly affect the hCG result. If a test manufacturer, for instance, uses a WHO-calibrated commercial hCG standard that contains significant nicked hCG, and the test does not detect nicked hCG, it will appear as if the assay gives lower results than it actually should (9,12,17). However, even if WHO standards were available in larger quantities, as stated earlier, they are based on urine-based material, which may be inappropriate as a calibrator for serum-based assays.

LIMITATIONS OF DETECTING PREGNANCY

Urine-Based Tests

One of the principal uses of an hCG assay is to aid in the early detection of pregnancy. Home pregnancy and point-of-care urine tests are often the first indication of pregnancy during the third, fourth, and fifth weeks following the last menstrual period. Most home pregnancy and point of care test devices have manufacturer claims of “over 99% accurate” and “use as early the first day of missing a period.” How valid are these claims?

Production of hCG does not begin until the blastocyst implants in the uterus. A study by Wilcox et al. reported that 10% of the 136 pregnancies they examined had not yet implanted by the first day of the missed menses (10). Therefore, the highest possible screening sensitivity for an hCG test on the first day of the missed menses is 90% (95% confidence interval [CI] 84–94%). By 1 wk after the day of the missed menses, the highest sensitivity was estimated to be 97% (95% CI 94–99%). In addition, once hCG production has begun, urine hCG concentrations vary greatly between individuals at the same gestational age. A recent study by our group showed that to detect 95% of pregnancies with urine tests the day of the missed period, and at days 1, 2, and 3 after missing menses, would require test detection limits as low as 12.4, 21, 35, and 58 mIU/mL, respectively (21). Although most urine point of care tests have claimed detection limits of 25–100 mIU/mL, we found that only 1 of 18 home pregnancy tests (First Response Early Result) had a detection limit as low as 12.5 mIU/mL (21). A urine concentration of 100 mIU/mL hCG was needed, together with extended incubation times beyond the time suggested by manufacturers for 18 of 18 devices to yield positive results. At a detection limit of 100 mIU/mL it was estimated that only 16% of pregnancies would be detected on the day of the missed menses (21). Based on these recent studies, we determined that a detection limit of 25 mIU/mL should detect 95% of pregnancies somewhere between 1 and 2 d after missing menses, and approx 74% of pregnancies at the time of missed menses (21).

How can manufacturers claim such a high accuracy in very early gestation? The answer relates to an arcane Food and Drug Administration (FDA) 510(k) regulation. The manufacturer needs to demonstrate only that its test results agree with those of an existing test more than 99% of the time in order to advertise “greater than 99% accuracy.” A new product is compared to an older FDA-approved test and evaluated with more than 100 urine samples supplemented with hCG at a concentration close to claimed sensitivity of the old test and with more than 100 urine samples containing no added hCG. The suggested use at the time of the missed menses and the 25 mIU/mL cutoff all come from studies performed with serum samples in the 1960s and 1970s and the now-proven erroneous assumption that serum and urine hCG concentrations are the same (3). These FDA guidelines and 510(k) evaluations, however, have no bearing on the ability of a product to detect early pregnancy. New guidelines are required for both home and point-of-care pregnancy tests that would require determination of the proportion of pregnancies detected by the product on a specific day of gestation (i.e., time of missed menses).

A word of caution should be given about the use of urine in general. Urine hCG concentrations can vary significantly depending on fluid intake. Therefore, a serum hCG-positive individual may test negative on a urine hCG test because of very dilute urine. This is one of the limitations of urine testing to confirm pregnancy, especially on random samples. A positive result is useful, but a negative result must be interpreted with caution.

Serum-Based Tests

Most quantitative serum hCG tests have a lowest detection limit of greater than 1 mIU/mL. Considering that there can be background hCG of pituitary origin in serum (22), most manufacturers recommend reporting negative results as less than 5 mIU/mL and using 5 mIU/mL as a cutoff for detecting pregnancy. Clearly, serum hCG tests are many times more sensitive than home pregnancy and point of care tests, and can detect more than 95% of pregnancies on or before the time of the missed menses. For this reason, quantitative serum hCG tests are the test of choice for pregnancy confirmation or for accurate early pregnancy detection.

Between one-quarter and one-third of naturally fertilized pregnancies and a much higher proportion of in vitro fertilized or assisted reproductive technology pregnancies fail to implant properly and result in early pregnancy losses. These lead to a transient increase in serum and urine hCG concentrations that diminish by the time of menses. An early pregnancy loss may also postpone menses 2 d (23). This transient production of hCG can potentially cause a so-called false-positive pregnancy test result with urine or serum tests at or around the time of the missed menses. This is referred to as a “biochemical pregnancy.” Repeat testing 2 to 3 d later will yield a true negative test result (13,23,24). Recent studies by O’Connor and colleagues indicate minimal hyperglycosylated hCG production by early pregnancy losses, and specific measurement of hyper-glycosylated hCG may avoid the false detection of early pregnancy losses (13).

In summary, it is clear that no test, serum or urine, can detect pregnancy with 99% accuracy at the first day of the missed menses. However, taking into account all of the above factors, it is estimated that urine or serum tests can detect pregnancy with 99% accuracy by 5 wk of gestation, or 1 wk following a missed menses.

LIMITATIONS OF hCG TESTS

Manufacturing Defects

Home pregnancy and point-of-care urine hCG tests are inexpensive disposable devices. Some poorly made devices can give false positive results in the absence of hCG, others can fail to function properly resulting in false negatives. These devices incorporate a test window and a control window. The test window indicates a positive or negative result and the control window indicates that the device is functioning properly (see “Principals of hCG Tests” above). Invalid tests fail to show a band in the control window. In a recent study examining home pregnancy tests (21), two devices (Confirm and Clear Choice home pregnancy tests) gave one or more false-positive results with a urine solution containing 0 mIU/mL hCG. Similarly, 9 of 30 tests using the Clear Choice and 10 of 30 tests using the Confirm were invalid because they lacked the proper formation of a band in the control window. It is important to confirm all home pregnancy and point-of-care urine test results with quantitative serum hCG tests. Qualitative and semi-quantitative serum point-of-care tests are calibrated and somewhat better controlled products. Although they offer an improvement over urine point-of-care products, they are still a “second best choice” and positive results need to be confirmed using a quantitative serum test.

The Hook Effect

The “hook effect” is a major limitation of all serum and urine one-step immunometric assays (see “Principals of hCG Tests” section). The hook effect occurs when extremely high concentrations of an analyte such as hCG occupies all the sites on both the capture and detection antibodies and prevents the formation of a so called “sandwich.” The end result is that few or no tracer antibody + hCG + immobilized-antibody complexes will be formed, yielding a false negative result. The “hook effect” does not occur with two-step quantitative assays because excess analyte is washed away before the tracer antibody is added. This problem has been documented for both quantitative and qualitative hCG assays (25–29). As a rule, if a physician finds that the hCG result is inconsistent with the clinical presentation (e.g., patient is clearly at 8–10 wk of pregnancy, and a quantitative hCG is 10 mIU/mL, or a point-of-care test is negative), the qualitative or quantitative test should be repeated with 10- and 100-fold diluted sample (9,22,30).

Heterophilic Antibodies

Heterophilic antibodies are human antibodies against other antibodies (human or animal) that can link a capture and tracer antibody in the absence of specific analyte and give a false-positive hCG result (30–33). Because antibodies are large glycoproteins and generally do not cross the glomerular basement membrane and enter urine, this is only a problem in serum, not urine, assays (30–33). The USA hCG Reference Service is a reference facility consulting with physicians in cases of conflicting or nonrepresentative hCG test results. In the last 5 yr, the USA hCG Reference Service has identified 54 cases of women who were erroneously treated for gestational trophoblastic disease, choriocarcinoma, or ectopic pregnancy because of false-positive hCG results (4,22,32–38). In the first few months of operation, the USA hCG Reference Service investigated three unusual cases (22). In all three cases, the women had an incidental pregnancy test that was positive. The positive hCG persisted with small apparent changes in concentrations. Ultrasound, dialation and curetlage, and laparoscopy ruled out pregnancy or ectopic pregnancy. The diagnosis of gestational trophoblastic disease or choriocarcinoma was assumed. In two of the three cases, chemotherapy was started, and in one case a hysterectomy was carried out. At this time, the reported hCG concentrations were 17, 53, and 110 IU/L, respectively. In all three cases, the presence of hCG was not confirmed and it was shown that these assays were subject to interference by heterophilic antibodies. To date, approx 145 individuals have been referred to the USA hCG Reference Service for investigating potential false positive hCG results. Fifty-four subjects were confirmed to have true false-positive results. False-positive results were identified by the following criteria (22,32–36):

1. the finding of more than a fivefold difference in serum hCG results when tested with an alternative immunoassay (critical criterion)
2. the presence of hCG in serum and absence of detectable hCG or hCG-related molecule immunoreactivity in a parallel urine sample (critical criterion)

Note: Where possible, urine testing should be performed using a quantitative hCG test with high sensitivity (sensitivity 2 mIU/mL). Even though this is an “off-label” application, it is the most appropriate and quickest confirmation of real or false-positive hCG. Point-of-care urine tests are limited to a sensitivity of 25 mIU/mL. We have found that

- a qualitative urine test is not certain to confirm a serum hCG result unless that result exceeds at least 100 mIU/mL. In addition, because most qualitative assays currently detect intact hCG, they will not detect free β -hCG in the urine in cases of β -hCG-producing germ cell tumor. Germ cell tumors that produce only free β chain can present a similar clinical picture as heterophile antibodies (i.e., unexpected positive quantitative serum hCG, no clinical signs of pregnancy, and a negative qualitative urine hCG test).
3. the observation of false-positive results in other tests for molecules not normally present in serum, such as urine β -core fragment (confirmatory criterion)
 4. the finding that a heterophilic antibody blocking agent (such as HBR, produced by Scantibodies Inc.) prevents or limits false positive (confirmatory criterion)

In all 54 confirmed false-positive cases, there was no prior history of trophoblastic disease or other tumors. Patients were treated for a diagnosis of ectopic pregnancy, gestational trophoblastic disease, or choriocarcinoma. Each case started with an incidental pregnancy test. Forty-five of 54 received needless surgery or single-agent chemotherapy; many received an unnecessary hysterectomy or other major surgery, or cytotoxic combination chemotherapy (32–38). To the best of our knowledge, in all cases, after false-positive hCG was identified, all treatment was halted, even though the quantitative test remained positive. Women having false-positive hCG results may also have falsely elevated results in other unrelated tests such as CEA, CA125, PSA, thyroid hormones, troponin, and other tumor and cardiac markers (39).

In most cases, false positive hCG results (in the USA hCG Reference Service assays) were eliminated by pretreatment of serum with a heterophilic antibody blocking agent, HBT (Scantibodies Inc., San Diego, CA) (36). It is noteworthy that certain hCG assays seem to have a propensity for producing false-positive results. All of the 53 false-positive cases arose from physicians who were monitoring patients with the Abbott AxSym hCG β assay, Bayer Centaur test, Bayer ACS180, Bayer Immuno-1, Roche Elecsys, J&J Vitros ECI, Tosoh Nexia, and Dade Dimension RXL quantitative serum tests or with the Beckman Icon 2 serum point of care hCG test. It is noteworthy that 43 of the 53 false-positive cases detected by the USA hCG Reference Service arose from centers monitoring patients with the Abbott AxSym hCG β assay. This has been observed by other centers (38). The Abbott AxSym test appears to be particularly prone to giving false-positive hCG results. An examination of the instruction sheet shows that animal serum is added in the Abbott AxSym test to just the diluent, rather than to the antibody preparation. As such, undiluted samples may not be protected from heterophilic antibody interference. This may explain the preponderance of false-positive results with this test (36,37).

Many of the clinicians who managed the 54 false-positive cases were misled by transient decreases in the hCG values after chemotherapy or surgery. This is because the decreases in hCG falsely suggested the presence of disease or indicated the success of therapy. The transient decreases were likely an interim weakening of the immune system after chemotherapy or surgery, reducing circulating heterophilic and antianimal antibody concentrations, leading to decreased false-positive hCG results.

SUMMARY

hCG is a complex glycoprotein that is produced by the trophoblastic cells of the placenta during pregnancy and in gestational trophoblastic diseases. Concentrations of hCG rise very rapidly during early pregnancy and peak at approx 8 and 12 wk. Numerous quantitative and qualitative assays for detecting hCG in serum or urine are available. All

of these assays are two-site “sandwich”-type immunoassays. Although immunoassays for hCG have existed for more than 25 yr, many problems still exist, causing considerable interassay variation. The problems can be traced to two fundamental factors: (1) differences in assay specificity, and (2) standardization material. Assay specificity problems are caused by the use of a variety of different antibodies that recognize any combination of the different molecular forms of hCG. To further compound the problem, good standardization material does not exist, and the material that does exist is not available in sufficient quantities. Pregnancy detection is itself problematical because of variation in the timing of initial hCG production by individuals, wide variation in the extent of hCG production, and hCG production by early pregnancy losses. Further limitations of the hCG assays themselves include manufacturing defects, the hook effect, and heterophilic antibodies. Considering these observations, great care is needed in the use and interpretation of pregnancy tests. Home pregnancy tests and point-of-care pregnancy tests should be considered as indicators of pregnancy, and confirmation of pregnancy should be obtained using a quantitative serum assay.

REFERENCES

1. Kovalesvskaya G, Birken S, Kakuma T, O'Connor JF. Early pregnancy human chorionic gonadotropin (hCG) isoforms measured by immunometric assay for choriocarcinoma-like hCG. *J Endocrinol* 1999;161:99–106.
2. Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated hCG (invasive trophoblastic antigen) immunoassay: a new basis for gestational down syndrome screening. *Clin Chem* 1999;45:2109–2119.
3. Butler SA, Khanlian SA, Cole LA. Detection of early pregnancy forms of hCG by home pregnancy test devices. *Clin Chem* 2001;47:2131–2136.
4. Cole LA, Shahabi S, Butler SA, et al. Utility of commonly used commercial hCG immunoassays in the diagnosis and management of trophoblastic diseases. *Clin Chem*, 2001;47:308–315.
5. Cole LA, Kohorn EI, Kim GS. Detecting and monitoring trophoblastic disease: new perspectives in measuring human chorionic gonadotropin levels. *J Reprod Med* 1994;39:193–200.
6. Kohorn EI, Cole LA. Nicked human chorionic gonadotropin in trophoblastic disease. *Intl J Obstet Gynecol* 2000;10:330–335.
7. Elliott M, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the α - and β -subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. *Endocrine* 1997;7:15–32.
8. Cole LA, Kardana A, Park S-Y, Braunstein GD. The deactivation of hCG by nicking and dissociation. *J Clin Endocrinol Metab* 1993;76:704–713.
9. Cole LA, Kardana A. Discordant results in human chorionic gonadotropin assays. *Clin Chem* 1992;38:263–270.
10. Wilcox AJ, Baird DD, Dunson D, McChaneay R, Weinberg CR. Natural limits of pregnancy testing in relation to the expected menstrual period. *J Am Med Assoc* 2001;286:1759–1761.
- 10a. Aswood ER. Evaluating health and maturation of the unborn: the role of the clinical laboratory. *Clin Chem* 1992;38:1523–1529.
- 10b. Aswood ER. Clinical chemistry of pregnancy. In: Burtis CA, Aswood EK, eds. *Tier Textbook of Clinical Chemistry*, 3rd ed. Philadelphia, Saunders, 1998;1736–1775.
11. Kovaleskaya G, Genbacev O, Fisher SJ, Caceres E, O'Connor JF. Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source of choriocarcinoma-like hCG. *Mol Cell Endocrinol* 2002;94:147–155.
12. Cole, LA. Immunoassay of hCG, its free subunits and metabolites. *Clin Chem* 1997;43:2233–2243.
13. O'Connor JF, Elish N, Kakuma T, Schlatterer J, Kovalevskaya G. Differential urinary gonadotrophin profiles in early pregnancy and early pregnancy loss. *Prenatal Diagnosis* 1998;18:1232–1240.
14. Storing PL, Gaines-Das RE, Bangham DR. International reference preparation of human chorionic gonadotrophin for immunoassay: potency estimates in various bioassay and protein binding assay

- systems; and international reference preparations of the α and β subunits of human chorionic gonadotrophin for immunoassay. *J Endocr* 1980;84:295–310.
15. Cole LA, Cermik D, Bahado-Singh R. Oligosaccharide variants of hCG-related molecules: potential screening markers for Down syndrome. *Prenat Diagn* 1997;17:1188–1190.
 16. Birken S, Berger P, Bidart J-M, et al. Preparation and characterization of new W.H.O. reference reagents for human chorionic gonadotropin (hCG) and metabolites. *Clin Chem* 2002;49:144–154.
 17. Sutton JM, Higgins TN, Cembrowski GS, Cole LA. Source of inter-assay variations in hCG test results. *Clin Chem* submitted 2003 (to be updated at proof).
 18. College of American Pathologists, Ligand Assay Series, CA-B 2002 Survey, 2002; p. 32.
 19. Bock JL. hCG assays: a plea for uniformity. *Am J Clin Path* 1990;93:432–433.
 20. Canfield RE, Ross GT. A new reference preparation of human chorionic gonadotrophin and its subunits. *Bull World Health Organization* 1976;54:463–72.
 21. Khanlian S, Davies S, Sutton JM, Cole LA, Rayburn WF. Utility of home pregnancy test kits at the time of missed menses. *Am J Obstet Gynecol*, submitted, 2004. In press.
 22. Cole LA. Phantom hCG and phantom choriocarcinoma. *Gynecol Oncol* 1998;71:325–329.
 23. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med* 1988;319:189–194.
 24. Bjercke S, Tanbo T, Dale PO, Morkrid L, Abyholm T. Human chorionic gonadotrophin concentrations in early pregnancy after in-vitro fertilization. *Human Reprod* 1999;14:1642–1646.
 25. Yoo A, Zaccaro J. Falsely low hCG level in a patient with hydatidiform mole caused by the “high-dose hook effect.” *Lab Med* 2000;31:431–435.
 26. Witherspoon LR, Schuler SE, Joseph GF, Baird GF, Neely HR, Sonnemaker RE. Immunoassay for quantifying choriogonadotropin compared for assay performance and clinical application. *Clin Chem* 1992;38:887–894.
 27. Forest JC, Masse J, Lane A. Evaluation of the analytical performance of the Boehringer Mannheim Elecsys 2010 immunoanalyzer. *Clin Biochem* 1998;31:81–88.
 28. Levavi H, Neri A, Bar J, Regev D, Nordenberg J, Ovadia J. “Hook effect” in complete hydatidiform molar pregnancy: a falsely low level of beta-hCG. *Obstet Gynecol* 1993;82:720–721.
 29. Stickle DF, Gronowski AM, Olsen GA, Fellows PA, Avery MB, Studts DJ. Decreased signal intensity of Sure-View serum/urine qualitative hCG test at very high [hCG]. *Clin Chem* 2000;46:A3.
 30. Hussa RO, Rinke ML, Schweitzer PG. Discordant human chorionic gonadotropin results: causes and solutions. *Obstet Gynecol* 1985;65:211–219.
 31. Vladutiu AO, Sulewski JM, Pudlak KA, Stull CG. Heterophilic antibodies interfering with radioimmunoassay: a false-positive pregnancy test. *J Am Med Assoc* 1982;248:2489–2490.
 32. Ward G, McKinnon L, Badrick T, Hickman PE. Heterophilic antibodies remain a problem for the immunoassay laboratory. *Am J Clin Path* 1997;108:417–421.
 33. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. *Lancet* 2000;355:712–715.
 34. Cole LA, Butler SA. False positive or phantom hCG result: a serious problem. *Clin Lab Intl* 2001; 25:9–14.
 35. Cole LA, Rinne KM, Shababi S, Omrani A. False positive hCG assay results leading to unnecessary surgery and chemotherapy and needless occurrence of diabetes and coma. *Clin Chem* 1999;45:313–314.
 36. Butler SA, Cole LA. The use of heterophilic antibody blocking agent (HBT) in reducing false positive hCG results. *Clin Chem* 2001;47:1332–1333.
 37. Cole LA, Butler SA. hCG, its free subunits and metabolites in trophoblastic diseases. *J Reprod Med* 2002;47:433–444.
 38. Olsen TG, Hubert PR, Nycum LR. Falsely elevated human chorionic gonadotropin leading to unnecessary therapy. *Obstet Gynecol* 2001;98:843–845.
 39. Covinsky M, Laterza O, Pfeifer JD, Farkas-Szallasi T, Scott MG. An IgM antibody to E. coli produces false positive results in multiple immunometric assays. *Clin Chem* 2000;46:1157–1161.
 40. Birken S, Armstrong EG, Kolks MAG, et al. Structure of human chorionic gonadotropin β -subunit core fragment from pregnancy urine. *Endocrinology* 1988;123:572–580.
 41. Morgan FJ, Birken S, Canfield RE. The amino acid sequence of human chorionic gonadotropin: the α subunit and β -subunit. *J Biol Chem* 1975;250:5247–5257.
 42. Laphorn AJ, Harris DC, Littlejohn A, et al. Crystal structure of human chorionic gonadotropin. *Nature* 1994;369:455–460.

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