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Review

The hCG assay or pregnancy test

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Abstract

This review examines human chorionic gonadotropin (hCG) or pregnancy tests from multiple perspectives. It first investigates the molecule hCG and shows that the term represents five independent molecules differing in carbohydrate and meric structure that share a common amino acid sequence. The review goes on to show that multiple degradation produces also the need to be tested for an hCG or pregnancy test to be optimally efficient. The review then carefully examines the literature showing the sensitivity and specificity of automated laboratory tests. Point-of-care pregnancy tests are then investigated along with over-the-counter pregnancy tests. Appropriate detection of hyperglycosylated hCG, nicked hCG, nicked hCG missing the β-subunit C-terminal peptide and nicked hyperglycosylated hCG is a limitation on all pregnancy tests. In the opinion of the author, just one automated laboratory test, the Siemen's Immulite, one point-of-care test, the Beckman-Coulter Icon 25, and one brand of over-the-counter device, First Response, are suitable for early pregnancy detection and possibly other applications.

Keywords: hCG; hCG test; hyperglycosylated hCG; pregnancy; pregnancy test.

Introduction

Here, we consider the human chorionic gonadotropin (hCG) assay or pregnancy test in the proper or most complete manner as has not been previously. To optimally examine the hCG assay we first consider what is hCG. hCG is a generic term applied to five individual, independent variants each sharing the amino acid backbone of hCG. These include hCG, the key pregnancy hormone, produced by syncytiotrophoblast cells during pregnancy. Hyperglycosylated hCG is the autocrine that controls pregnancy implantation and placental growth during pregnancy. Sulfated hCG, the molecule produced by gonadotrope cell during the female menstrual cycle, is the hormone that supplements luteinizing hormone (LH) during multiple applications. Finally, there are two hCGβ variants that are critical to most cancers and their biologies, hCGβ and hyperglycosylated hCGβ. These two cancer promoters drive most advanced malignancies through growth and invasion or malignancy. As such, all hCG tests or pregnancy tests need to detect at the very least these five hCG primary molecules. This review starts by examining the prevalence of these five critical molecules.

Associated with placental syncytiotrophoblast and cytotrophoblast cells are monocytes, leukocytes, macrophages and placental Hofbauer cells that produce a leukocyte elastase that starts hCG and its five variants on degradation pathways immediately upon secretion (1). In this review we carefully examine all these degradation products of hCG. For a total hCG test to be called appropriately a total hCG test it need to be able to detect the five hCG variants and their degradation products. Here, we review these two issues before examining the specificity of different hCG tests. First we examine laboratory total hCG tests, then point-of-care (POC) office hCG tests and finally over-the-counter (OTC) home pregnancy tests.

To be complete, this review first examines what is hCG. This part of the review looks at the five independent hCG-related molecules. Second the review examines hCG intermediates. Here, taking the hormone hCG as an example, this section looks at the dissociation and degradation intermediates in the clearance of hCG. Third, considering the molecules present in the circulation and urine of patients, this review examined the laboratory use of total hCG tests. Fourth, point-of-care or office hCG tests are examined and finally over-the-counter or home hCG tests are investigated.

The author acknowledges a potential conflict of interest. Since 2004 the author has been a paid advisor for Church and Dwight Inc. As all the over-the-counter testing described in this review was performed blindly (testers had no knowledge of which urine should be positive or of the make of devices tested), the author claims no bias.

What is hCG?

hCG is an oddball molecule. There actually are five biologically active variants of hCG, produced by different cells and having different functions. Some hCG-related molecules are hormones and others are autocrines or intra-cellular signaling

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molecules, they all have very different structures. We ask, why do all these independent molecules share the name hCG? These molecules, while having a common amino acid backbone structure, are all different overall in structure (Figure 1). This is because these molecules are 28%–42% glycosylated (Table 1). The hCG series of molecules are the most glycosylated glycoproteins known. In 1997, we discovered hyperglycosylated hCG (2). In the 4 years that followed I called it invasive trophoblast antigen to differentiate it from hCG (6, 7). In 2002, I received a letter from the World Health Organization, telling me that since it has the same amino acid sequence as hCG it had to be named as a form of hCG, including the term hCG. In 2003 I named it hyperglycosylated hCG. Thus, all five independent molecules were named as hCG variants, hCG, hyperglycosylated hCG, sulfated hCG, hCGβ and hyperglycosylated hCGβ (Figure 1).

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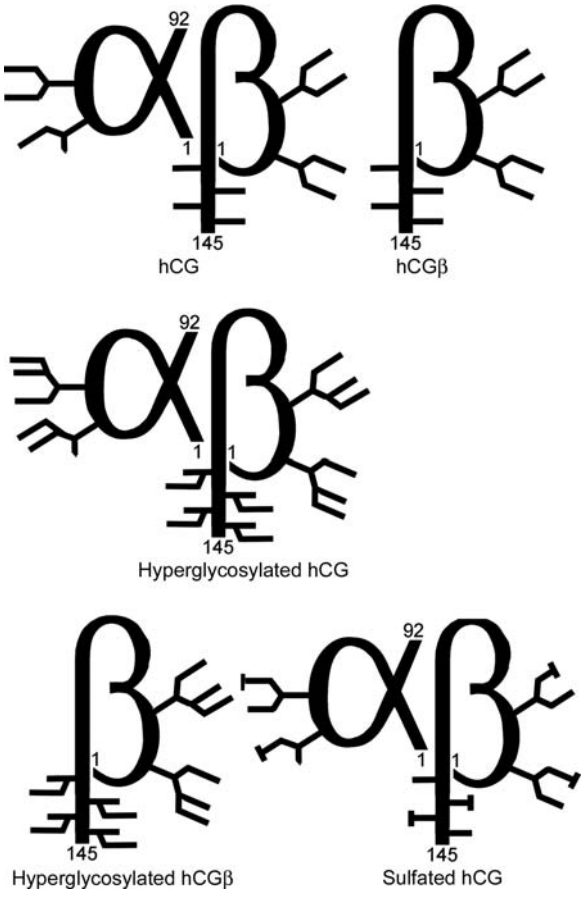

Figure 1 Comparative structures of the five different bioactive forms of hCG.
The oligosaccharide structures are shown as monoantenarry, biantennary and triantennary structures to represent the monoantennary O-linked and biantennary N-linked structures on regular glycosylated molecules and biantennary O-linked and triantennary N-linked structures on hyperglycosylated molecules. O-linked structures are placed close to residue 145 to represent C-terminal peptide. The comparative structures do not show folding or three dimensional structures, which for all molecules except hCG remain unknown. Sulfated oligosaccharides are shown as slightly shorter structures.

Figure 2 illustatrates the structures of the four N-liked and four O-linked oligosaccharides on hCG, sulfated hCG, hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ. As indicated, hCG and its free β-subunit, hCGβ, contain biantennary N-linked oligosaccharides and simple trisaccharide O-linked oligosaccharides. Occasionally, tetrasaccharide O-linked oligosaccharides are seen with two NeuAc or sialic acid residues (2, 4). Sulfated hCG oligosaccharides terminate in either sialic acid (NeuAc) linked to galactose (Gal), yielding the terminal structure – NeuAc α2,3 Gal β1,4 GlcNAc or sulfated (SO_4) N-acetylgalactosamine (GalNAc), yielding the terminal structure SO_4-GalNAc β1,4 GlcNAc (Figure 2). As published by Birken et al. (3), sulfated hCG contains on average 3.4 moles of sulfate terminating structures to 6.3 moles of sialic acid structures, suggesting that most oligosaccharides are sialylated. Hyperglycosylated hCG and hyperglycosylated hCGβ contain large triantennary N-linked oligosaccharides and double size hexasaccharide O-linked oligosaccharides (Figures 1 and 2).

As noted, the five variants of hCG are produced by separate cells (Table 1) and have separate functions. hCG is the hormone produced in placental fused cells, syncytiotrophoblast cells (8). The glycosylation of regular hCG is shown in Figure 2. hCG has a wide range of function during pregnancy, it acts on a joint luteinizing hormone (LH)/hCG receptor promoting cyclic AMP production. On the maternal side, hCG promotes progesterone production by corpus luteal cells for the first 3–4 weeks of gestation (3–7 weeks gestation) (9). During pregnancy, hCG promotes angiogenesis in uterine cells so that uterine arteries reach the invading placenta (10). On the other side, hCG promoted formation of an umbilical and umbilical circulation (11). While hyperglycosylated hCG promotes cytotrophoblast cell growth (12, 13), hCG promotes it fusion to syncytiotrophoblast cells (14) and villous trophoblast tissue formation, the cells at the maternal-fetal circulation interface. This combination of hyperglycosylated hCG and hCG action, assessing the uterine spiral arteries and the umbilical circulation, together with villous placenta formation, leads to hemochorial placentation or efficient passage of nutrients to the fetus (15).

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hCG has multiple other functions affecting uterine growth and fetal growth. hCG inhibits phagocytosis or maternal macrophage and immune system destruction of the genetically foreign invading feto-placenta unit (16). hCG promotes uterine growth parallel to fetal growth (17). hCG also suppresses uterine contraction during the term of pregnancy (18). Most interestingly, recent research indicates, with the finding of hCG/LH receptors, that hCG promotes growth and differentiation of fetal organs during pregnancy (19, 20). In essence, hCG promotes most major actions during pregnancy, hCG should be renamed the pregnancy hormone.

Hyperglycosylated hCG is a super-glycosylated variant of hCG, with double size O-linked oligosaccharides and 1.5× N-linked oligosaccharides (Figure 2). Hyperglycosylated hCG is produced by root placenta cells, cytotrophoblast cells (8). Hyperglycosylated hCG functions with hCG as an autocrine by promoting growth of cytotrophoblast cells (13, 21–23). Hyperglycosylated hCG also drives cytotrophoblast cell

Table 1 The properties of the five independent variants of hCG.

Parameter	hCG	Sulfated hCG	Hyperglycosylated hCG	hCGβ	Hyperglycosylated hCGβ
Source cell of synthesis	Syncytiotrophoblast	Gonadotrope	Cytotrophoblast	Advanced malignancies	Advanced malignancies
Mode of action	Endocrine	Endocrine	Autocrine	Autocrine	Autocrine
Site of action	LH/hCG receptor	LH/hCG receptor	TGFβ antagonism	TGFβ antagonism	TGFβ antagonism
Components:					
1. Amino acids α-subunit	92	92	92	–	–
2. Amino acids β-subunit	145	145	145	145	145
3. O-linked sugar units	4	4	4	4	4
4. N-linked sugar units	4	4	4	2	2
Peptide molecular weight	26,200	26,200	26,200	16,000	16,000
Molecular weight sugars	10,980	9950	16,600	7300	11,600
Total molecular weight	37,180	36,150	42,800	23,300	27,600
Percentage sugars	30%	28%	39%	31%	42%
Metabolic clearance rate	36 h	20 h	Not known	0.72 h	Not known

The amino acid content, molecular weight and sugar contents are as described by Elliott et al. (2) for hCG and hyperglycosylated hCG, by Birken et al. (3) for sulfated pituitary hCG and by Valmu et al. (4) for hyperglycosylated hCGβ. The molecular weight of common hCG dimer amino acid backbone is that as determined by Morgan et al. (5). Molecular weights are recalculated in this table using the amino acid sequences of Morgan et al. (5) and the carbohydrate structures of Elliott et al. (2) and Valmu et al. (4).

invasion causing implantation of a blastocyst in pregnancy (13, 21–24). Hyperglycosylated hCG also drives choriocarcinoma, persistent hydatidiform mole malignancies, testicular germ cell and ovarian germ cell malignancies (13, 21). Hyperglycosylated hCG seemingly functions very differently to hCG, acting as a transforming growth factor-β (TGFβ) antagonist, blocking cytotrophoblast cell apoptosis and driving collagenase and metalloproteinases production (12).

Sulfated hCG is made by pituitary gonadotrope cells during the menstrual cycle (3, 25–27). On sulfated hCG, NeuAc-α2,3-Gal- sugar residues, are replaced by SO_4-GalNAc- sugar residues (Figure 2) (3, 25–27). Gonadotrope sulfated hCG production parallels LH production during the menstrual cycle. It is thought that sulfated hCG supplement LH is promoting androstenedione and progesterone production and in causing ovulation (3, 25–27). Sulfate hCG levels in blood and urine are very low, approximately 1/50th LH levels, considering, however, that sulfated hCG is 50-times more potent than LH (3), the two hormones may have equal functions.

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hCG free β-subunit (hCGβ) and hyperglycosylated hCGβ are produced by most advanced malignancies. They are autocrines like hyperglycosylated hCG and antagonize a TGFβ receptor like hyperglycosylated hCG. These are the cancer promoters in advanced malignancies, driving cancer growth and invasion (12, 28, Cole, Cancer, submitted). Acting on this receptor, these promoters block cancer cell apoptosis and promote cancer cells to produce collagenases and metalloproteinases (12, 28–33). hCG-forms are both pregnancy hormones and the root promoters that control cancer.

hCG intermediates

Placental trophoblast tissue, the site of production of hCG, is normally a "degradation grounds". As shown by the slide in Figure 3, 8 weeks villous placental tissue contains multiple monocytes, leukocytes, adjacent macrophage cells and Hofbauer cells or placental macrophage cells, making the tissue a "degradation grounds". Degradation continues in the circulation, kidney and liver with proteases and glycosidases. Macrophages, monocytes, leukocytes and Hofbauer cells all produce human leukocyte elastase, the enzyme that rapidly nicks hCG, hyperglycosylated hCG and hCGβ (1, 34–43) and cleaves the C-terminal peptide on these molecules.

As shown in Table 2, hyperglycosylated hCG (dissociation half-life 120 h) is dissociated twice as quick as hCG (dissociation half-life 240 h). Once hCG or hyperglycosylated hCG is nicked by leukocyte elastase, it much more rapidly dissociates (dissociation half-lives 44 and 22 h). As such, a high proportion of hCG, and particularly hyperglycosylated hCG, are rapidly processed to nicked free subunits (44, 45). With these pathways ongoing, measurement of hCG in serum or urine is an incomplete story without also measuring all these degradation and nicking products (Figures 4 and 5). It is only by assessing hCG plus its degradation products that a complete patient hCG story can be assessed. Here, we review hCG degradation pathways and assess what really needs to be measured and how it is measured, to assess the complete hCG picture.

As shown in Table 2, leukocyte elastase rapidly cleaves or nicks hCGβ and more slowly nicks hCG (1, 35). As published, six pregnancy urines averaged 30% nicking, while five purified hyperglycosylated hCG preparations from choriocarcinoma patient urine averaged 56% nicking (2). Leukocyte elastase nicks hCG on the β-subunit between residues β44 –45 or residues β47–48 (1, 2, 35, 37). Slowly, leukocyte elastase also cleaves or removes the C-terminal peptide of the β-subunit of hCG by cleavage at β92–93 (1, 2, 27, 35). It is inferred that all molecules missing the C-terminal peptide are also nicked, the more rapid the action of leukocyte elastase (1, 2, 27, 35).

hCG and hCGβ
N-linked oligosaccharides

Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,

NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,

Fuc α1
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1, 6
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,

O-linked oligosaccharides

NeuAc α2,3 Gal β1,4 GlcNAc-O-Ser

Sulfated hCG
N-linked oligosaccharides

NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,

SO_4-GalNAc β1,4 GlcNAc β1,2 Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,

SO_4-GalNAc β1,4 GlcNAc β1,2 Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
SO_4-GalNAc β1,4 GlcNAc β1,2 Man α1,

O-linked oligosaccharides

NeuAc α2,3 Gal β1,3 GlcNAc-O-Ser

SO_4-GalNAc β1,4 GlcNAc-O-Ser

Hyperglycosylated hCG and HyperglycosylatedhCGβ
N-linked oligosaccharides

Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,4 Man α1,
NeuAc α2,3 Gal β1,4 GlcNAc β1,2

NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1,
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,4 Man α1,
NeuAc α2,3 Gal β1,4 GlcNAc β1,2

Fuc α1
NeuAc α2,3 Gal β1,4 GlcNAc β1,2 Man α1, 6
6
3 Man β1,4 GlcNAc β1,4 GlcNAc-N-Asn
NeuAc α2,3 Gal β1,4 GlcNAc β1,4 Man α1,
NeuAc α2,3 Gal β1,4 GlcNAc β1,2

O-linked oligosaccharides

NeuAc α2,3 Gal β1,4 GlcNAc β1,6
NeuAc α2,3 Gal β1,3 GlcNAc-O-Ser

Figure 2 The N-linked and O-linked oligosaccharides on hCG, sulfate hCG, hyperglycosylated hCG, hyperglycosylated hCGβ and hCGβ as shown by Elliott et al. (2), Birken et al. (3) and Valmu et al. (4).

Leukocyte elastase also cleaves the N-terminal of β-subunit at β5–6, leading to the loss of residues 1–6 (2). Other protease cleave the α-subunit at α2–3 and α3–4. All the cleavages are illustrated in Figures 4 and 5. As published, most hCG degradation products are cleared through the liver (43). Molecules invariably lose sialic acid in the circulation, most notably in choriocarcinoma cases and in the third trimester of pregnancy (2, 36). Loss of sialic acid leads to rapid clearance by the liver (43).

hCG is first nicked, then either rapidly dissociates to become nicked hCGβ and loses the C-terminal peptide or loses

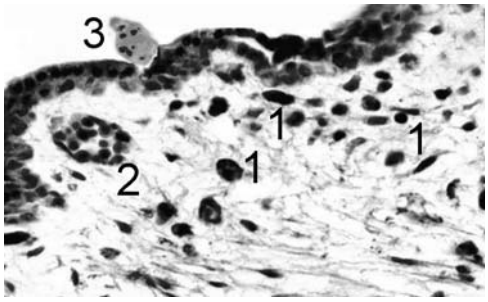

Figure 3 Placenta floating villous, 8 weeks gestation, stained with antibody B204 (binds β-subunit, nicked β-subunit, β-subunit missing C-terminus and β-core fragment).
The villi contain numerous monocytes stained from associated hCG cleavage products (1) as well as a placental macrophage (Hofbauer cell complex) (2). An associated macrophage is shown attached to syncytiotrophoblast cells (3).

the C-terminal peptide as nicked hCG and then dissociates to nicked hCGβ missing the C-terminal peptide (Figure 6). It has been determined that 78% of molecules are cleared from the circulation through the liver (43), this includes hCG, nicked hCG, nicked hCG missing the C-terminal peptide or nicked hCGβ missing the C-terminal peptide. Just 22% of molecules are cleared through the urine. In the kidney, nicked hCGβ missing the C-terminal peptide is degraded by

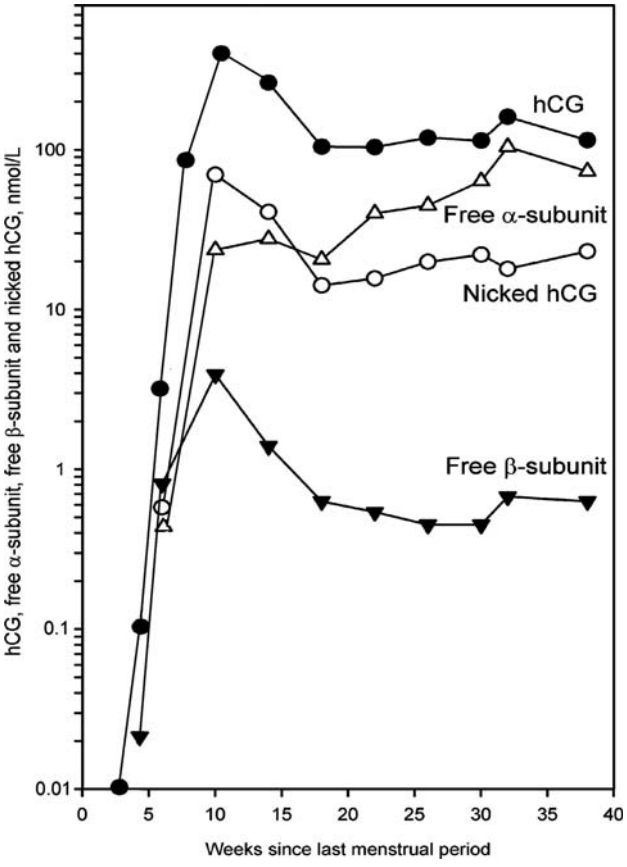

Figure 4 hCG and hCG degradation products in 465 pregnancy serum samples.

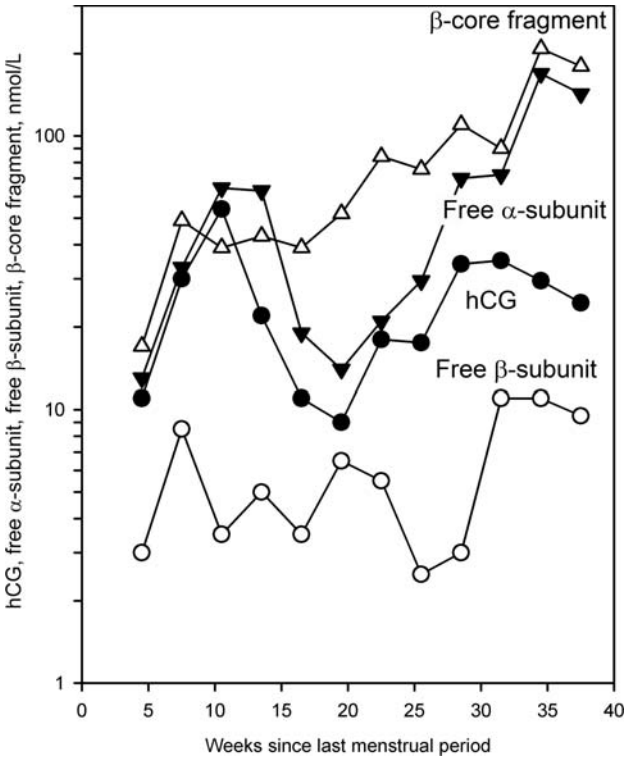

Figure 5 hCG and hCG degradation products in 546 urine samples during pregnancy.

exoproteinases and exoglycosidases to the β-core fragment, the terminal degradation product of hCG and its β-subunit (Figures 4 and 5).

hCGβ, as produced by cancer cells, is degraded in a similar rapid manner, it is first nicked and then nicked hCGβ loses the C-terminal peptide. Most molecules are cleared from circulation through the liver (43). Just a small percentage of molecules are cleared through the kidney as β-core fragments, the terminal degradation product (Figure 6). Acevedo and Hartstock (46) and Regelson (47) claim that 100% of advanced malignancies produce hCGβ, yet only 30% of all cancers tested in serum have detectable hCGβ (48). The reason for this inconsistency is obvious, the rapid degradation and clearance of hCGβ. As indicated in Table 2, hCGβ is very rapidly nicked by leukocyte elastase. It is reasonable to say that all serum hCGβ is probably nicked. As indicated in Table 2, hCGβ is also rapidly cleared from the circulation by the liver and kidney. This is why we do not detect it in serum. Urine β-core fragment is a slightly better general tumor marker that serum hCGβ (48% detection of all cancers) (48).

Hyperglycosylated hCG is degraded by a parallel pathway to hCG (Figure 6). The exception is that hyperglycosylated hCG is dissociated more rapidly than hCG and cleaved by elastase more rapidly than hCG (Table 2). Hyperglycosylated hCG is either nicked, dissociates or loses the C-terminal peptide (Figure 6). Alternatively, dissociated hyperglycosylated hCGβ is nicked and loses the C-terminal peptide. Biologically, hyperglycosylated hCG is quite different to hCG. hCG loses all biological activity upon nicking or upon dissociation to hCGβ (1, 49, 50). Hyperglycosylated hCG is an antagonist of the TGFβ receptor (12), so is nicked hyperglycosylated hCG and hyperglycosylated hCGβ (12, 49, 50). As such, while hCG dissociation and degradation products are inactive, hyperglycosylated hCG dissociation and degradation products may remain active.

Figure 4 examines hCG degradation products in serum during the course of pregnancy. As shown, hCG, free α-subunit, nicked hCG and free β-subunit are each specifically detected. A total of 465 individual serum samples were tested. Individual variation is very great. In pregnancy, nicked hCG and free α-subunit can become the principal molecule detected. To ascertain the true or complete hCG story for a specific patient it is important to measure all hCGβ-related molecules. In urine, for much of pregnancy, β-core fragment may be the principal hCG-form detected (Figure 5). Once again to complete the hCG story it is crucial to the detection of all hCGβ-related molecules.

Laboratory total hCG

As we learn from understanding that hCG is five independent molecules, hCG, hyperglycosylated hCG, hCGβ,

Table 2 Criteria leading to the dissociation and degradation of hCG-related molecules (1–4, 13, 19, 20).

1. Time of dissociation of dimer in serum in the laboratory at 37°C.	
hCG	Dissociation half-time 240 h
Hyperglycosylated hCG	Dissociation half-time 120 h
Nicked hCG	Dissociation half-time 44 h
Nicked hyperglycosylated hCG	Dissociation half-time 22 h
2. Effect of leukocyte elastase at 37°C	
hCG, 5 nmol, 1 h incubation	30% nicked at β44–45 and β47–48
β-subunit, 5 nmol 1 h incubation	100% nicked at β44–45 and β47–48
3. Time for injected molecules in humans to leave the circulation	
hCG	Fast phase half-time 5.97 h, slow phase half-time 36 h
β-subunit	Fast phase half time 0.68 h, slow phase half-time 3.9 h
α-subunit	Fast phase half time 0.22 h, slow phase half-time 1.3 h
Asialo hCG	Fast phase half-time 0.060 h, slow phase half-time 0.096 h
β-core fragment	Fast phase half-time 0.058 h, slow phase half-time 0.38 h

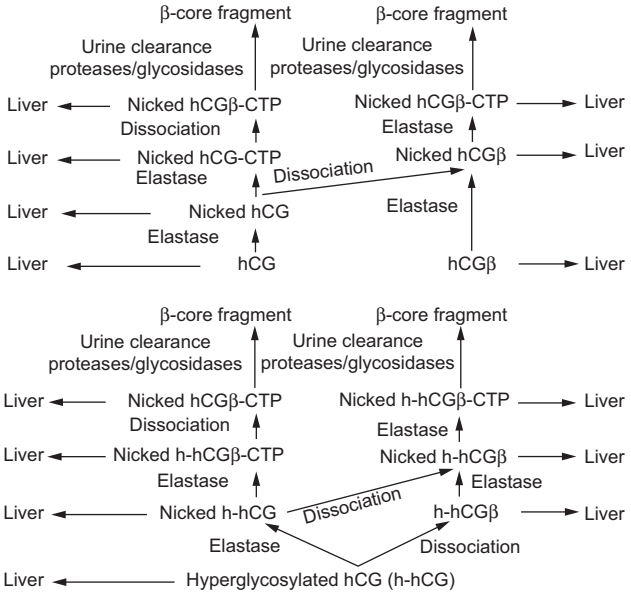

Figure 6 Established pathways for the degradation and clearance of hCG, hCGβ (1, 2, 34–43) and hyperglycosylated hCG.

hyperglycosylated hCGβ and sulfated hCG. Unfortunately no standard is available for sulfated hCG or hyperglycosylated hCGβ, to evaluate all laboratory automated total hCG tests. We assume that sulfated hCG is recognized by total hCG test parallel to hCG and that a test which appropriately detects hCGβ and hyperglycosylated hCG will detect hyperglycosylated hCGβ. We also learn from degradation studies that standards are needed for nicked hCG, nicked hCGβ, nicked hyperglycosylated hCG and at least nicked hCG missing the β-subunit C-terminal peptide. We first considered using the WHO standards. Unfortunately their standards have a purity problem. Firstly, the pure hCG standard contains a nicked hCG and hyperglycosylated hCG component (2). Secondly, their nicked hCG contains a hyperglycosylated hCG component, and so on. Furthermore, WHO has no standard for hyperglycosylated hCG, the most important molecule to test after hCG, and no standard for nicked hyperglycosylated hCG or nicked hCG missing the β-subunit C-terminal peptide.

We resorted to using our own standards. We purified molecules from five pregnancy, five hydatidiform mole and five choriocarcinoma patient urines (2). Each preparation was completely purified. To complete the story, the peptide and N-linked and O-linked oligosaccharide structure were all determined (2). While we could not identify a pure hCG that was not contaminated by nicked moles and hyperglycosylated molecules, we found a pure 0% nicked and a pure 100% nicked 100% hyperglycosylated hCG, a 100% nicked hCG and a nicked hCG missing the β-subunit C-terminal peptide, very unique and special standards for testing hCG assays (2). For pure hCG and pure hCGβ we used commercial CHO-cell recombinant hCG and hCGβ preparations. All standards were diluted in normal male serum and then blindly evaluated in all 11 automated total hCG immunoassay systems at 13 laboratories in the USA, New Zealand and Canada. The results are presented in Table 3 (51). It should be noted

Table 3 Specificity of automated laboratory total hCG tests (51).

Serum standards	Abbott Architect	Abbott AxSYM	Beckman Access 2	Beckman DxI 800	Ortho Vitros ECiQ	Roche Elecsys hCG+β	Siemens ACS180	Siemens Centaur	Siemens Dimension	Siemens Immulite	Siemens Stratus	Tosoh A1A
hCG, %	96	103	103	100	112	109	105	104	96	96	92	95
Hyperglycosylated hCG, %	86	85	120	98	68	78	102	81	67	105	66	nd
Nicked hCG, %	70	99	84	71	80	69	85	66	65	115	8	nd
Nicked hCG missing CTP, %	0	0	0	0	0	12	0	0	10	109	28	16
Nicked hyperglycosylated hCG, %	40	46	46	51	80	100	70	40	80	103	88	70
Asialo hCG, %	35	69	48	46	85	46	81	39	65	114	73	59
hCGβ, %	87	94	142	136	47	102	126	47	47	111	73	66
Nicked hCGβ, %	33	51	56	63	19	53	72	19	41	107	70	60
β-core fragment, %	1	1	1	1	1	16	0	1	1	35	1	1

Results are percentage values, this is nmol/L as determined in the assay as a percentage of the absolute concentration of tested standard. βCTP is β-subunit C-terminal peptide. nd, not determined.

Q5: Please confirm expansion for nd.

that 12 automated immunoassay systems are described. Many of the systems are marketed in median production models (i.e., Siemens Immulite 1000), high production models (i.e., Siemens Immulite 2000) and fast turn-around high production models (i.e., Siemens Immulite 2500). In these cases only one model is evaluated. Since they all use the same antibodies, comparable specificities are expected. In all, automated hCG assays were tested with (Table 3) Serum hCG, Serum hyperglycosylated hCG, Serum nicked hCG, Serum nicked hCG missing the β-subunit C-terminal peptide (abbreviated CTP in Table 3), Serum nicked hyperglycosylated hCG, Serum asialo hCG, Serum hCGβ, Serum nicked hCGβ and Serum β-core fragment (normally only exists in urine).

As found (Table 3), hCG variants were detected variably in all 12 assay systems. The test performing the best was the Siemens Immulite, appropriately detecting all standards. One standard that was detected poorly by the Siemens Immulite (standard result more than 25% different from standard concentration) was β-core fragment, which was only detected with 35% efficiency. In my opinion, this assay can be used for (i) 6 weeks to term pregnancy and (ii) 3–6 week gestation early pregnancy screening, (iii) cancer and (iv) gestational trophoblastic disease detection and (v) monitoring hCG clearance post-parturition or termination. All the 11 other hCG tests were seemingly limited by poor detection of nicked hCG missing the β-subunit C-terminal peptide. This was because they seemingly used a C-terminal peptide antibody as a capture or tracer antibody. This excluded the use of 11 or 12 test systems in (iii) cancer and (iv) gestational trophoblastic disease detection and (v) monitoring hCG clearance post-parturition or termination. These assays can only complete two of five hCG functions.

As found (Table 3), three assay systems, Ortho Vitros ECiQ, Siemens Dimension and Siemens Stratus poorly detected hyperglycosylated hCG, and six assays poorly detected nicked hyperglycosylated hCG, a common variant of hyperglycosylated hCG, the Abbott Architect, Abbott AxSym, Beckman Access-2, Beckman DXI800, Siemens ACS 180 and Siemens Centaur. This only left two assay systems, other than the Siemens Immulite, suitable for early (3–6 weeks of gestation) pregnancy screening or standard pregnancy testing (hyperglycosylated hCG is the predominant form of hCG present at 3–6 weeks gestation). These tests are the Roche Elecsys and Tosoh A1A systems. The Roche Elecsys, Siemens Centaur and Siemens Stratus systems fail because of poor detection of nicked hCG, and the Beckman Access 2, Ortho Vitros ECiQ, Siemens ACS 180 and Siemens Centaur, Siemens Dimension, Siemens Stratus and Tosoh A1A systems fail because of inappropriate detection of hCGβ.

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The USA hCG Reference Service has also had the opportunity to evaluate the test from a further perspective, frequency of report of false-positive total hCG results (52). The least number of reports in the last 5 years (zero reports) came with the Abbott Architect and Abbott AxSym assays. Rare reports (one report) of false-positive cases have been observed with the Tosoh A1A, Siemens ACS180 and Roche Elecsys assays (52). Frequent false-positive cases were reported in the last 5 years with the other assays. Abbott may be fairing well at this time. However, Abbott was the cause of a large false-positive problem with the Abbott AxSym, 1998–2003 (53).

Multiple microtiter plate immunometric assays are available to test most hCG-related antigens. Table 4 lists the antibodies used by the USA hCG Reference Service for detecting nicked hCG, hyperglycosylated hCG, hCGβ and β-core fragment, total hCG, molecules with a C-terminal peptide and hyperglycosylated hCGβ. All of the antibodies are available commercially or from other scientists.

Point-of-care hCG tests

We have cautiously evaluated the specificities, sensitivities and abilities of some of the more commonly used POC pregnancy tests, dip stick type tests, to detect pregnancy (54). We examined six commonly used POC tests. Each of these tests are a representation of the test currently used at physician's offices, prenatal care clinics and clinical laboratories. As shown in Table 5, the POC devices vary widely in sensitivity for hCG, with sensitivities ranging from 12–50 IU/L. The Quidel QuickVue and the Beckman Coulter Icon 25 devices equally detected regular and hyperglycosylated hCG; however, the Mainline Maxie and Confirms, Inverness Accreava hCG Basic II, and the Siemens Clinitest tests poorly detected hyperglycosylated hCG relative to hCG (Table 5). In early pregnancy at 3–6 weeks of gestation, hyperglycosylated hCG is the predominant form of hCG present in serum and urine, so a test that poorly detects hCG-H is not appropriate for testing pregnancy at this time, the most common period women are tested for pregnancy. None of the POC devices tested detected free β-subunit, a third product produced in early pregnancy.

We tested the abilities to detect pregnancy on the day of missing of menses or approximately 4 weeks of gestation (54). The Beckman Coulter Icon 25 test had equal detection of regular hCG and hyperglycosylated hCG and faired the best at detecting day of missing menses pregnancies (Tables 5 and 6). This test had the highest sensitivity, detecting 77% of pregnancies on the day of missing menses (Table 6). The Quidel QuickVue, which also equally detects regular hCG and hyperglycosylated hCG, faired reasonably and detected 67% of pregnancies on the day of missing menses (Table 6). The other four POC devices tested (Siemens, Clinitest Digital, Inverness Acceava, Mainline Maxie and Mainline Confirms) poorly detected hyperglycosylated hCG (Table 1) and detected just 43%, 67%, 50% and 40% of pregnancies on the day of missing menses, respectively (Table 6). All POC tests claim >99% accuracy and claim use as early as 3 days prior to the day of missing menses. The results of this study suggest that the >99% claims are erroneous and confusing as are the claim to detect pregnancy 3 days prior to the day of missing menses.

The hook effect is a problem with POC tests. The hook effect occurs when an anti-α core antibody in the POC device is combined with an anti-β-subunit antibody. Excess amounts of free α-subunit in urine and serum samples can completely occupy the α-subunit antibody binding site, blocking any region for hCG to bind. This leads to a negative pregnancy result. The hook effect can occur at any point during pregnancy. Similarly, if tests use a β-subunit core antibody a hook effect can occur. β-core fragment can predominate in urine

Table 4 Combinations of antibodies used in microtiter plate immunometric assays as run by the USA hCG References Service.

Capture antibody	Tracer antibody	Specificity	Purpose
B108	INN-hCG-22	hCG, nicked hCG, nicked hCG-CTP, hCGβ, nicked hCGβ, nicked hCGβ-CTP, hyperglycosylated hCG, nicked hyperglycosylated hCG, nicked hyperglycosylated hCG-CTP, β-core fragment	Detects all hCGβ molecules
2119	H222	hCG, nicked hCG, nicked hCG-CTP, hyperglycosylated hCG, nicked hyperglycosylated hCG-CTP	Only detects dimer
B152	H222	Hyperglycosylated hCG, nicked hyperglycosylated hCG, hyperglycosylated hCGβ, nicked hyperglycosylated hCGβ	Only detects hyperglycosylated
B151	H222	Nicked hCG, nicked hCGβ, nicked hyperglycosylated hCG and nicked hyperglycosylated hCGβ	Only detects nicked
FBT11	H222	hCGβ, nicked hCGβ and hyperglycosylated hCGβ	Free β only, no CTP
B210	H222	β-core fragment	β-core fragment only
CCF01	H222	hCG, nicked hCG, hyperglycosylated hCG, nicked hyperglycosylated hCG, hCGβ, nicked hCGβ	Molecules with CTP only
B152	B204	Hyperglycosylated hCGβ, nicked hyperglycosylated hCGβ	Hyperglycosylated hCGβ only
B151	B204	Nicked hCGβ, nicked hyperglycosylated hCGβ	Nicked free β
2090–1114	2119	hCGα, pituitary free α	Free α assay

Tracer antibodies are all peroxidase-labelled. The abbreviation "-CTP" refers to molecules missing the β-subunit C-terminal peptide.

and fully occupy one of the antibody sites which can cause a false-negative test. This problem primarily occurs during the 2nd and 3rd trimesters of pregnancy (55).

Over-the-counter pregnancy tests

OTC pregnancy tests are a first line device offered for sale in pharmacies, food stores and general stores for home use. We evaluate here the six most commonly purchased brands of devices, the Church and Dwight Inc. First Response Manual and First Response Gold Digital tests, the Pfizer Consumer Healthcare Inc. EPT Manual and EPT Certainty Digital tests and the Inverness Medical Innovation Inc. Clear Blue Easy Manual and Clearblue Easy Digital devices. There are numerous generic and other devices marketed that are manufactured by Inverness Medical Innovation Inc. that have comparable sensitivities and specificities to the Inverness

Table 5 The sensitivity and specificity of point-of-care pregnancy tests.

Device	hCG standard, mIU/L	Hyperglycosylated hCG standard, molar mIU/mL equivalents	Free β-subunit standard, molar mIU/mL equivalents
	Sensitivity	Sensitivity	Sensitivity
Quidel QuickVue One-Step	24	24	88
Beckman Coulter Icon 25	12	12	88
Siemens Clinitest, Digital Analyzer	50	100	>500
Inverness Acceava hCG Basic II	18	50	>500
Mainline Maxine hCG	24	50	>500
Mainline Confirms hCG	24	50	>500

Six devices were tested at each of six concentrations, in molar equivalents of hCG (first reference reagent): 6, 12, 24, 50, 100 and 500 mIU/mL. In each evaluation (35 tests) the lowest concentrations leading to six of six positive results, or sensitivity, is reported. For example, for the Quidel QuickVue One-Step evaluation of hCG Standard, six devices were tested at 500 mIU/mL, six at 100 mIU/mL, six at 50 mIU/mL, six at 24 mIU/mL, six at 12 mIU/mL and six mIU/mL. Six of six were positive at 500 mIU/mL, 100 mIU/mL, 50 mIU/mL and 24 mIU/mL. At 12 mIU/mL and 6 mIU/mL invariable numbers of the six devices or none were positive. It is concluded that the sensitivity is the lowest concentration at which six of six were positive, of 24 mIU/mL.

Table 6 Evaluation of point-of-care tests with 30 urine samples from women on the day of missing their menstrual period (calculated from lengths of three previous menstrual cycles).

Total hCG, IU/L	Quidel QuickVue One-Step	Beckman Coulter Icon 25	Siemens Clinitest	Inverness Acceava Basic II	Mainline Maxie hCG	Mainline Confirms hCG
41	1	1	1	0	0	0
142	1	1	1	1	0	0
56	1	1	1	1	1	1
179	1	1	1	1	1	1
138	1	1	1	1	1	1
24	0	0	0	0	0	0
103	1	0	0	0	1	0
61	1	1	0	1	0	0
31	0	0	0	1	0	0
224	0	0	0	0	0	0
34	1	1	0	1	0	0
176	0	1	0	1	0	0
18	0	1	0	0	0	0
68	1	1	1	1	1	1
135	1	1	1	1	1	1
5.8	0	0	0	0	0	0
89	0	0	0	0	0	0
33	1	1	0	1	1	0
8.9	0	0	0	0	1	0
260	1	1	1	1	1	1
85	1	1	0	1	0	0
103	1	1	1	1	1	1
32	0	1	0	1	0	0
150	1	1	1	1	1	1
122	1	1	1	1	1	1
173	1	1	0	1	1	1
31	0	1	0	0	0	0
147	1	1	1	1	1	1
37	1	1	0	1	0	0
196	1	1	1	0	1	1
Mean	67%	77%	43%	67%	50%	40%

Devices were run exactly as specified by Manufacturer (Siemens device measured in digital device analyzer), 0 indicates negative and 1 equals a positive device.

Clearblue Easy Digital devices. Church and Dwight Inc. produce a compatible device called Answer, with similar affinities to First Response.

In this article we examine the sensitivity and specificity of the six most commonly purchased devices, blindly, without any preference for the device being tested, and very thoroughly. The six trials or studies presented here have all been published (56). Five of the trials were performed without financial backing, the sixth study was financed by Church and Dwight Inc. (56). However, all studies show very compatible results, consistent with blind analysis.

In the first trial (Table 7), the six OTC devices were tested with six concentrations of hCG. The First Response Manual test had a sensitivity of 3.3 mIU/mL, First Response Digital of 5.5 mIU/mL and the two EPT devices had sensitivities of 11 mIU/mL, as did the two Clearblue easy devices. In the second trial (Table 7), hyperglycosylated hCG, the early pregnancy antigen, was recognized with equal affinity by the First Response devices. The manual EPT and Clearblue Easy devices had equal affinity between hCG and hyperglycosylated hCG, but the digital devices had 2-fold lower affinity for hyperglycosylated hCG. While in trial 3, the First Response devices detected free β-subunit, the EPT and Clearblue Easy devices did not. In Trial 4, all devices were tested with a mixture of hCG, hyperglycosylated hCG and free β-subunit, comparable with that in urine at 5–6 weeks gestation. The two First Response devices showed an overall sensitivity of 5.5 mIU/mL with the mixture, while the two EPT and two Clearblue Easy devices showed an overall sensitivity of 22 mIU/mL (Table 7). In conclusion, the two First Response devices show a 4-fold greater sensitivity that the competing devices, EPT and Clearblue Easy.

Devices were then blindly evaluated with early pregnancy urines (Table 8). In trial 5, devices were examined with 40 blinded individual urines collected on the day of missing menses and 3 days after the day of missing menses. The First Response Manual test had a sensitivity of 100% on the days of missing menses and 100% 3 days later. The First Response Digital test had a sensitivity of 98% and 95%. The EPT manual test had sensitivities of 55% and 80%, the EPT Digital test of

Table 7 Evaluation of over-the-counter tests with urine mixtures containing pure hCG, pure free β-subunit and pure hyperglycosylated hCG standards.

Concentration	First Response Manual	First Response Gold Digital	EPT Manual	EPT Certainty Digital	Clearblue Easy Manual	Clearblue Easy Digital
Trial 1. Non-pregnant female urine containing hCG dimer 1st RR standard						
55 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
38 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
22 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
11 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
5.5 mIU/mL	6 of 6	6 of 6	2 of 6	2 of 6	3 of 6	2 of 6
3.3 mIU/mL	6 of 6	4 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	3.3	5.5	11	11	11	11
Trial 2. Non-pregnant female urine containing C5 hyperglycosylated hCG standard						
55 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
38 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
22 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
11 mIU/mL	6 of 6	6 of 6	6 of 6	2 of 6	6 of 6	4 of 6
5.5 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
3.3 mIU/mL	6 of 6	2 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	3.3	5.5	11	22	11	22
Trial 3. Non-pregnant female urine containing free β-subunit 1st RR standard						
90 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
63 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
36 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
18 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
9 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	9.0	9.0	>90	>90	>90	>90
Trial 4. Non-pregnant female urine containing 40% hCG, 40% hyperglycosylated hCG, 20% free β-subunit combination						
55 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
38 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
22 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
11 mIU/mL	6 of 6	6 of 6	4 of 6	1 of 6	2 of 6	3 of 6
5.5 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	5.5	5.5	22	22	22	22

Concentration of standards are molar equivalents of hCG in mIU/mL.

65% and 80%, the Clearblue Easy Manual test of 58% and 75% and the Clearblue Easy Digital test of 60% and 75% (Table 8).

In a much larger trial, 80 individual early pregnancy urines were evaluated daily over 11 days, from 6 days prior to the missed menses, until 4 days after the missed menses, or 80×11 or 880 evaluations (summarized in Table 8). The First Response Manual test detected 25% of pregnancies 6 days prior, 74% of pregnancies 3 days prior, 96% of pregnancies on the day of missing menses and 100% of pregnancies 3 days later. The First Response Digital test detected 25% of pregnancies 6 days prior, 68% of pregnancies 3 days prior, 96% of pregnancies on the day of missing menses and 100% of pregnancies 3 days later. The EPT manual test detected 0% of pregnancies 6 days prior, 14% of pregnancies 3 days prior, 53% of pregnancies on the day of missing menses and 80% of pregnancies 3 days later. The EPT Digital test detected 0% of pregnancies 6 days prior, 18% of pregnancies 3 days prior, 68% of pregnancies on the day of missing menses and 86% of pregnancies 3 days later. The Clearblue Easy Manual test detected 0% of pregnancies 6 days prior, 27% of pregnancies 3 days prior, 67% of pregnancies on the day of missing menses and 87% of pregnancies 3 days later. The Clearblue Easy Digital test detected 0% of pregnancies 6 days prior, 12% of pregnancies 3 days prior, 51% of pregnancies on the day of missing menses and 84% of pregnancies 3 days later (Table 8).

Clearly, from Trials 5 and 6, the only home pregnancy test that detects 100% or all pregnancies on the day of missing menses is First Response Manual. All of the other home pregnancy tests need to catch up with this requirement. That home pregnancy tests now advertize use as early as 4–6 days prior to missing the menses is totally ridiculous. Since at this time only 29% (6 days prior), 40% (5 days prior) and 76% (4 days prior) of pregnancies have implanted or officially started. Even if they have implanted it does not constitute a 100% clinical pregnancy because biochemical pregnancies still occur. In our experience, biochemical pregnancies truly account for 39% of implantation events (Cole LA, Fertil Steril, manuscript submitted). Manufacturers should stop competing trying to offer earlier and earlier home pregnancy tests, since what is the point of an OTC pregnancy tests if all pregnancies have not implanted? A woman who tests herself for pregnancy 6 days prior to missing menses many not realize the meaninglessness

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Table 8 Evaluation of over-the-counter tests with pregnancy urines.

Day relative to missed period	Achieved pregnancy, %	hCG median, mIU/mL	First Response Manual, %	First Response Gold Digital, %	EPT Manual, %	EPT Digital, %	Clearblue Easy Manual, %	Clearblue Easy Digital, %
Trial 5. Pregnant women, 40 total								
0	100	41	100	98	55	65	58	60
+3	100	154	100	95	80	80	75	75
Trial 6. Pregnant women, 80 total								
–6	29	2.1	25	25	0	0	0	0
–5	40	2.9	33	25	5.0	5.0	5.0	5.0
–4	76	5.2	58	42	6.3	6.3	8.8	3.8
–3	88	12	74	68	14	18	27	12
–2	92	21	76	81	29	31	29	28
–1	99	40	93	91	42	55	57	51
0	99	70	96	96	53	68	67	51
+1	100	143	100	96	64	71	74	69
+2	100	227	100	99	77	79	81	77
+3	100	302	100	100	80	86	87	84
+4	100	534	100	100	100	100	100	100

of the test (only 29% of pregnancy have implanted). Clearly, the First Response tests are the most sensitive pregnancy tests. This article suggests the use of 100% sensitive home pregnancy tests at the optimal time, on the day after missing menses, when all pregnancies have implanted (Table 8).

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Choice of serum and urine hCG tests

In the USA, the Food and Drug Administration dictates that hCG immunoassays can only be used quantitatively for examining serum samples. Urine testing is limited to POC hCG tests and OTC hCG tests, and to qualitative determination by laboratory hCG immunoassays. Quantitative urine hCG testing by immunoassay is available in many countries outside of the USA. We know that many centers outside the USA utilize urine hCG testing. We are told that the Charing Cross Hospital Trophoblast Disease Center in London, for instance, finds urine quantitative urine measurement as useful as serum measurement in managing gestational trophoblastic disease cases. This is probably very true. We ask here what are the advantages and limitations of serum and urine hCG

Table 9 The advantages and limitations of serum and urine hCG measurements.

Urine	Serum
Advantages	*Advantages*
1. Represents all hCG forms produced, whether they are forms like hCGβ or hCG missing the βC-terminal peptide, that may be cleared very rapidly from the circulation or hCG itself, cleared slowly from the circulation.	1. Is a stable and constant fluid medium and does not vary with liquid intake.
2. hCG is degraded rapidly during clearance of hCG, following parturition, abortion of pregnancy, evacuation of hydatidiform mole or ectopic pregnancy, residual hCG or the last remnants of hCG clearance may only be in urine.	2. Serum has constant pH and serum containing hCG can be frozen and thawed without significant loss of hCG.
3. In a person having heterophilic antibodies interfering with Serum measurements, only a urine hCG assay is meaningful.	
Limitations	*Limitations*
1. Urine is invariably diluted by liquid intake. This can be normalized by measuring urine creatinine.	1. hCG and its variants and degradation intermediates are all cleared from serum at dramatically different clearance rates. As such serum only gives the hCG production picture at the second in time that the phlebotomy was performed, rather than the overall picture of relative amounts produced.
2. Urine sample varies in pH among individuals.	2. Heterophillic antibodies interfere with serum measurements.
3. The kidney can cleave hCG forms. hCG-free β that is missing the β C-terminal peptide, for instance, can be cleaved further by exopeptidases and glycosidases in the kidney, such that only β- core fragment is present in the urine.	
4. As reported by Stenman and Alfhan (58), urine hCG can change greatly with freezing at –20°C and thawing.	

tests (57)? As listed in Table 5, serum and urine tests have different advantages as well as multiple limitations.

As listed in Table 5, there are advantages and limitation to both urine and serum hCG measurement (57). Serum values are more constant and are not as affected by liquid intake as urine results can be. Serum values, in contrast, can become meaningless due to serum hetrophilic antibodies, interfering antibodies that can cause a false-positive hCG assay. While hCG is cleared from the circulation slowly, its dissociation product hCGβ may be cleared rapidly. As such serum results much more represent a point of time result and not what is produced, while urine results may be more representative of what is produced. All told, for qualitatively detecting pregnancy, either serum testing or urine testing is appropriate. For a quantitative assessment of advancement of a pregnancy, or for detecting hCG in cancer or pregnancy disorder, testing hCG may be most appropriate.

Readers need to understand that when considering using an hCG or pregnancy test you must first realize that the term hCG refers to a compilation of five different highly heterogeneous variants. Only two of these variants reflect on pregnancy, the hormone hCG and the autocrine hyperglycosylated hCG. Both exist as multiple charged forms and are present in the circulation as a mixture of intact and dissociated (hCGβ, hyperglycosylated hCGβ) and degraded forms (nicked hCG, nicked hCG missing the β C-terminal peptide, nicked hCGβ, nicked hCGβ missing the β C-terminal peptide, variably sialylated hCG and hCGβ, hyperglycosylated and variably sialylated hyperglycosylated variants of these four degradation). One of these variants, sulfated hCG, is produced by the pituitary and three of these variants, hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ, are produced by different cancers. If you are strictly interested in pregnancy, the field is open to automated laboratory hCG tests, POC hCG tests and OTC hCG tests. If you are are strictly interested in pregnancy and only in measuring the un-dissociated and un-degraded intact hormone hCG, then any of the automated tests, POC hCG tests and OTC hCG tests described in this review may be appropriate. If you are interested in measuring hCG and all its degradation products, to give a total picture of everything produced in pregnancy, I recommend the automated Siemens Immulite total hCG test. This is the only automated laboratory test that can equally detect all hCG variants in the circulation. Similarly, if your interest is pituitary hCG or any form of cancer, I also recommend the Siemens Immulite assay in that this is the only current test which detects all determinants.

Conflict of interest statement

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Q14: Please update Ref. 58

Laurence A. Cole PhD is an hCG person. His PhD 30 years ago was on production of hCG β-subunit and cancer. His first faculty position was at University of Michigan, in Ann Arbor. From that point his multiple major grants on hCG and cancers led to him being solicited by Obstetrics and Gynecology at Yale University. After 14 years at Yale University he was solicited as a full tenured professor at the University of New Mexico Medical School to head the new division of Women's Health Research. At the University of New Mexico he opened the USA hCG Reference Service. In this position he became the only endowed professor as the Howard and Friedman Distinguished Professor of Obstetrics and Gynecology. Professor Cole PhD is world renowned as a specialist in the hCG field, with 34 years experience working with hCG, 300 publication featuring hCG, the discovery and patenting of hCG free β and cancer, the discovery and patenting of hyperglycosylated hCG, the discovery of hyperglycosylated hCG and Down Syndrome, the discovery and patenting of hyperglycosylated hCG and hyperglycosylated hCGβ as cancer markers and antibodies to hyperglycosylated hCG in cancer treatment, of quiescent gestational trophoblastic disease, of familial hCG syndrome, of minimally aggressive gestational trophoblastic neoplasm, of the role of hCG in human evolution and many more patents and discoveries.