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Total hCG tests

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ABSTRACT

Introduction: There are 12 types of automated total hCG tests sold today, the Abbott Architect, Abbott AxSym, the Beckman Access 2, Beckman Dxl 800, the Ortho Vitros EciQ, Roche Elecsys hCG+β, Siemens ACS180, Siemens Centaur, Siemens Dimension, Siemens Immulite and Siemens Stratus, and the Tosoh A1A. All tests claim to be total hCG tests but do not define what total means. Total hCG test needs to detect all hCG variants in order to be used for all hCG test clinical applications. Here we assess this ability.

Methods: Coded samples of pure hCG, nicked hCG, hyperglycosylated hCG, nicked hCG missing C-terminal peptide, nicked hyperglycosylated hCG, asialo hCG, hCGβ, nicked hCGβ and β-core fragment were tested blindly in serum and urine at 10 independent laboratories.

Results: While the Siemens Immulite total hCG test detected 8 of 9 hCG variant standards, other assays poorly detected important determinants such as nicked hCG missing the C-terminal peptide, β-core fragment, hyperglycosylated hCG, nicked hCG, asialo hCG, and hCGβ. Four assay appropriately detected 4 of 9 variants, 2 assays detected 3 of 9, 4 assays detected 2 of 9 and 1 assay only appropriately detected 1 of 7 hCG variants.

Discussion: Care is needed in selecting a total hCG test. The Siemens Immulite tests performed best at detecting all the hCG variants making it appropriate for all applications. Nine assays had limited applications, 3 of the assays were appropriate for advanced pregnancy testing only.

1. Introduction

Automated total hCG assays have become the standard for hCG testing. Although hCG tests today are named "total hCG tests" manufacturers of assays do not specify which hCG variants or hCGβ-related variants are detected by their assay. In 2004 we evaluated multiple automated hCG tests. At that time, assays were found to vary from detecting all hCG variants to just measuring hCG [1]. Here we re-examine all total hCG automated tests of 2011. It is critical that the specificity of assays to different hCG variants be reported so that the full application of a test is known. Detecting all forms of hCG is essential for all total hCG applications. In all cases, a total hCG test is ordered by a physician, with no knowledge about the limitation of different tests for specific applications. For this reason it is clinically important that all tests detect all hCG forms.

Firstly, it should be noted, that 4 independent hCG-related molecules, each with separate biological function [2,4]. These are hCG made by syncytiotrophoblast cells. Hyperglycosylated hCG made by cytotrophoblast cells but having much larger sugar side chains than hCG, hCGβ or hyperglycosylated hCGβ, made by all advanced malignancies. Sulfated hCG, made by the pituitary gonadotrope cells. A total hCG test needs to, at the least, equally detect hCG, hyperglycosylated hCG, hCGβ and sulfated hCG.

In early pregnancy, at 3–5 weeks of gestation, the principle hCG molecule produced is hyperglycosylated hCG [4,5] (Table 1). If a test does not appropriately detect hyperglycosylated hCG it cannot be rightfully called an early pregnancy test. Hyperglycosylated hCG is also the principal form of hCG produced in invasive mole and choriocarcinoma cases [6,7] (Table 1). hCGβ, nicked hCGβ, nicked hCGβ missing the C-terminal peptide and hyperglycosylated hCGβ are the principal forms of hCG detected in serum in non-trophoblastic malignancy cases [8,9] (Table 1). Both hCGβ and hyperglycosylated hCGβ are critical in detecting Down syndrome pregnancies [10] (Table 1). Nicked hCG missing the C-terminal peptide is evident during clearance after ectopic pregnancy, spontaneous abortion and parturition [12], and both hCGβ and nicked hCG missing the C-terminal peptide are the solitary hCG-related molecules produced in Familial hCG syndrome [13] (Table 1). Measurement of nicked hCG missing the C-terminal peptide is important in monitoring women with gestational trophoblastic diseases (Table 1, Fig. 1). hCG molecules can vary greatly during normal and abnormal pregnancy in sialic acid content and the detection of all sialylated molecules from asialo hCG to fully sialylated hCG [14] (Table 1). Measurement of urine β-core fragment is important in pregnancy, gestational trophoblastic

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Table 1
Multiple hCG-related molecules, occurrence and biological function [2,6,9–13]. βCTP is β-subunit C-terminal peptide.

Molecule	Relationship to total hCG	Detection is serum and urine
hCG	Independent molecule with separate biological functions. It promotes progesterone production, uterine angiogenesis during pregnancy, uterine quiescence, umbilical cord growth, fetal growth, and uterine growth during pregnancy [1].	1. Produced during the length of pregnancy
Hyperglycosylated hCG	Independent molecules, separate biological functions. It promotes invasion as in implantation and growth of cytotrophoblast cells [6].	1. Principal hCG molecule in early pregnancy 2. Elevated in trisomy pregnancies 3. Principal molecule produced in choriocarcinoma, gestational trophoblastic neoplasm, and persistent hydatidiform mole. 4. Critical in Down syndrome screening
Nicked hCG	hCG cleaved by macrophage or leukocyte enzymes such as elastase, cleavage at β47–48. Biologically inactive degradation product of hCG.	1. Present is pregnancy serum and urine. 2. Significant component of hCG following evacuation of an ectopic pregnancy or spontaneous abortion, or parturition.
Nicked hyperglycosylated hCG	Hyperglycosylated hCG is rapidly nicked by macrophages of leukocyte enzymes such as elastase, cleavage at β47–48 [10].	1. Principal molecule produced in choriocarcinoma, gestational trophoblastic neoplasm, and persistent hydatidiform mole.
Nicked hCG missing βCTP	Further cleavage of nicked hCG by macrophage or leukocyte enzymes such as elastase. C-terminal peptide is cleaved from β-subunit at 92–93. Biologically inactive degradation product of hCG [10].	1. Commonly detected in serum and urine of patients with hydatidiform mole or choriocarcinoma. 2. Detected in serum and urine following evacuation of an ectopic pregnancy or spontaneous abortion, or parturition. 3. Produced in Familial hCG syndrome.
Asialo hCG	Recombinant hCG standard incubated with Neuraminidase to remove sialic acid.	1. hCG with variable sialic acid content produced in pregnancy and gestation trophoblastic disease. Standard hCG has full complement of sialic acid, this has none representing other extreme of normality.
hCGβ	Dissociation product of hCG and independent molecule with separate biological functions produced by cancer cells. It promotes growth and invasion of cancer cells producing this molecule [8] [13].	1. Dissociation product is critical in early pregnancy and in Down syndrome screening. 2. hCGβ or hyperglycosylated hCGβ produced by most advanced malignancies, detected as β-core fragment in urine. 3. Detected in Familial hCG syndrome.
Nicked hCGβ	hCGβ cleavage enzymes such as elastase, cleavage at β47–48. Rapid cleavage occurs in blood. Degradation product of hCGβ [8,10,13].	1. Nicked hCGβ or nicked hyperglycosylated hCGβ present in serum and urine of most cases with advanced malignancies.
β-core fragment	A urine terminal degradation product of nicked hCG missing C-terminal peptide. β-core fragment comprises hCGβ residues 6–40 disulfide linked to residues 55–92 [10].	1. β-core fragment detected in urine of most cases with advanced malignancies. 2. Detected in urine following evacuation of an ectopic pregnancy or spontaneous abortion, or following parturition.

disease and cancer testing. β-core fragment is the urine terminal degradation product of hCG, hyperglycosylated hCG and hCGβ [11,12].

Figs. 1,2 and 3 illustrate the importance of detection of all form of hCG in total hCG assays. Figs. 1 and 2 illustrate the need for detection of nicked hCG and nicked hCG missing the C-terminal peptide in abnormal pregnancy (Fig. 1: hydatidiform mole case; Fig. 2: spontaneous abortion of pregnancy case). As illustrated the assay not detecting nicked hCG yield unduly low results, while the test not detecting nicked hCG missing the C-terminal peptide yielded exceptionally low deceptive results. Fig. 3 illustrates the need for appropriate detection of hyperglycosylated hCG detection in monitoring early pregnancy.

Considering this data [1–15], it is important for hCG tests to detect nicked hCG, hyperglycosylated hCG, nicked hCG missing the C-terminal peptide, hCGβ, asialo hCG and β-core fragment, not just for routine pregnancy testing, but for all applications of hCG tests

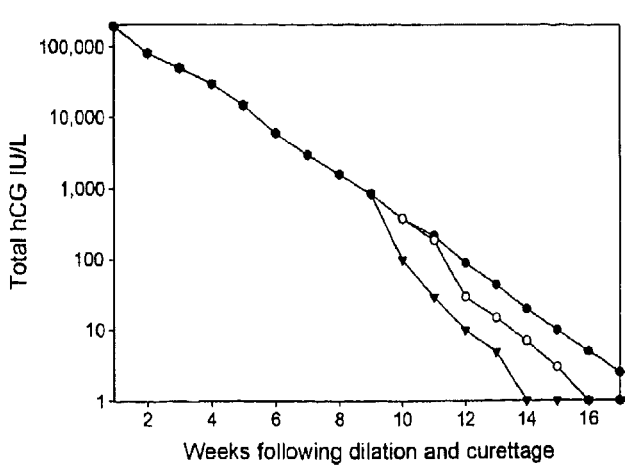

Fig. 1. Example of limitation of assay poorly detecting nicked hCG and nicked hCG missing the β-C-terminal peptide, following weekly the dilation and curettage of a complete hydatidiform mole. Black circles are the Siemens Immulite 1000 total hCG assay. Open circles are the AxSym total hCG assay (does not appropriately detect nicked hCG missing the C-terminal peptide). Black triangles are the B109 hCG assay (does not appropriately detect nicked hCG).

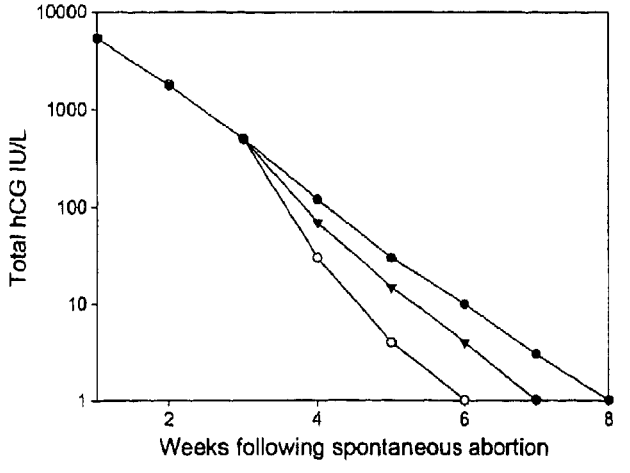

Fig. 2. Example of limitation of assay poorly detecting nicked hCG and nicked hCG missing the β-C-terminal peptide, following weekly the spontaneous abortion of pregnancy in the first trimester. Black circles are the Siemens Immulite 1000 assay. Open circles are the AxSym total hCG assay (does not appropriately detect nicked hCG missing the C-terminal peptide). Black triangles are the B109 hCG assay (does not appropriately detect nicked hCG).

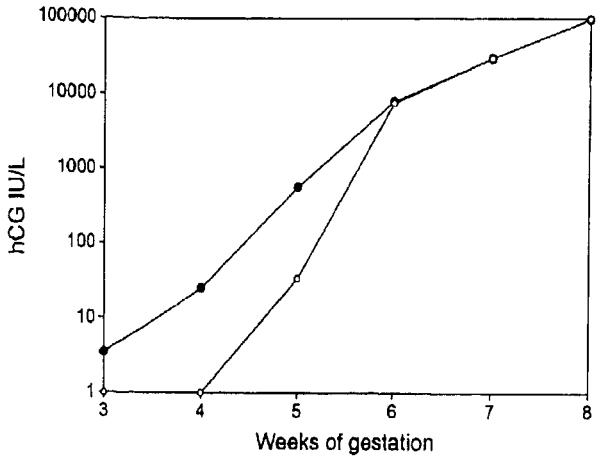

Fig. 3. Example of limitation of assay poorly detecting hyperglycosylated hCG, following weekly the advancement of a normal term pregnancy. Black circles are the Immulite 1000 assay. Open circles are the Serono MAIAclone assay (does not appropriately detect hyperglycosylated hCG).

including abnormal pregnancies, and cancer cases, monitoring the disappearance of hCG following ectopic pregnancy and spontaneous abortion, monitoring cases with gestational trophoblastic disease, or for all applications of hCG tests.

2. Methods

hCG related molecules and variants tested were all pure fully characterized (amino acid and carbohydrate structure) preparations [6]. We considered using World Health Organization standards for this study [18], but unfortunately, they do not have a hyperglycosylated hCG preparation nor a preparation of nicked hCG missing the C-terminal peptide, and their preparations are invariably not pure. Their hCG standard contains invariable amounts, 5–20% of hyperglycosylated hCG and 5–20% nicked hCG making them inappropriate for this study. We used CHO-cell recombinant hCG and hCGβ preparations, which are neither nicked nor hyperglycosylated (Table 2). CHO-cell recombinant hCG is the purest form of hCG of established structure [16,17].

Non-nicked hyperglycosylated hCG was purified from JEG-3 choriocarcinoma cells [19], which is the only cell line that produces 100% hyperglycosylated hCG without hCG or hCGβ [19]. Nicked hyperglycosylated hCG (100% hyperglycosylated, 100% nicked) was batch C5 [5] (Table 2). The nicked hCG used was batch M4 (100% nicked at β47–48) [6]. The nicked hCGβ used was the dissociation product of nicked hCG batch M4. Nicked hCG missing the C-terminal peptide was batch M2B (nicked at β47–48 and cleaved at β92–93) [6]. The β-core fragment used was batch P9 [5]. Asialo hCG was made by treating CHO-cell recombinant hCG with neuraminidase (3×24-hour incubation) using the described procedures [6].

The objective of this project was to determine the relative concentrations of these standards detected in automated hCG assay units. The standards tested were all pure as demonstrated by N-terminal sequence analysis [6], since they vary in molecular weight they were all calibrated in molar units, nmol/l or pmol/ml [1,20] (Table 2). Standards were all prepared and diluted in human male serum, and in urine for testing.

Samples were blindly coded and sent to the following laboratories for testing—Health Waikato Laboratories, Waikato Hospital, Hamilton, New Zealand (Siemens Immulite 2000 total hCG and Elecsys hCG+β); DynaLifeDx, Edmonton, AB, Canada (Abbott Architect total hCG, Abbott AxSym total hCG and Siemens Centaur total hCG); Tosoh Bioscience, Inc., San Francisco, CA (Tosoh A1A total hCG); Endocrine Laboratory, Yale University, New Haven CT (hCGβ Radioimmunoassay); Red Deer Regional Hospital Centre, Red Deer AB, Canada (Siemens Dimension Vista total hCG); Sturgeon Community Hospital, St Albert AB, Canada (Beckman Access 2 total hCG); University of Alberta Hospital, Edmonton AB, Canada (Beckman DxI800 total hCG); Wainwright Health Centre. Wainwright AB, Canada (Ortho Vitros EciQ total hCG); Northern Lights Health Centre Laboratory, Fort McMurray AB, Canada (Siemens Stratus total hCG). No tests were evaluated at Dr. Cole's laboratory, the USA hCG Reference Service.

3. Results and discussion

There are 12 automated total hCG tests sold today, Architect, AxSym, Access 2, DxI 800, Vitros EciQ, Elecsys hCG+β, ACS180, Centaur, Dimension, Immulite and Stratus, and A1A. Each type of test is sold for multiple machine versions, varying in throughput and automation but using the same antibodies and principals (i.e., Immulite 1000, 2000 and 2500). All claim to be total hCG tests but do not define what total means. Generally, total mean hCG plus hCGβ, but the word total implies all forms of hCG. Detection of all forms is needed, however, to cover all hCG applications, multiple hCG-related diseases, cancer and pregnancy disorders produce primarily hCG variants (hCGβ, hCG missing the C-terminal peptide, nicked hCG or hyperglycosylated hCG). In 2004 we evaluated multiple automated hCG tests. At that time, assays were found to vary from detecting all hCG variants to detecting just hCG [1]. We re-examine all total hCG automated tests as used in 2011.

Standards of hCG related molecules and variants that appear commonly in normal and abnormal pregnancy serum and urine and in cancer cases were tested with all 12 types of automated total hCG assays used in laboratories. For this study all standard were blindly coded and tested (Table 2). Results for the different assays were determined and decoded. Results were then divided by the actual concentration of the standards (concentration of standards in molar units, as listed in Table 2). Results were expressed as percentage reactivity (Table 3). Percentage values are the result in nmol/l expressed as a percentage of the actual concentration of the standard (Table 2). For comparison, results were also determined using a classic nineteen eighties hCGβ RIA. LH cross-reactivity in assays was also determined as was utility to detect hCG and hCGβ in urine samples (Table 3). Inappropriate test results, varying from equi-molar reactivity by more than 25% are highlighted (Table 3).

Each test was assessed for detecting advanced pregnancy (6 weeks gestation-term), for detecting early pregnancy (3/4/5 weeks gestation), for detecting abnormal pregnancy (ectopic pregnancy, spontaneous abortion, placenta accreta pregnancy, spontaneous abortion pregnancy, hydatidiform mole pregnancy), for Down syndrome screening, and for monitoring a non-trophoblastic cancer. For advanced pregnancy detection only appropriate detection of hXG was required. For early pregnancy detection, appropriate detection of hyperglycosylated hCG was mandatory. For abnormal pregnancy and non-trophoblastic cancer detection, detection of molecule missing the

Table 2
Calibration of hCG-related molecule standards in nmol/l (or pmol/ml) as used for assessing all total hCGβ tests. All preparations were pure [5]. βCTP is β-subunit C-terminal peptide.

Molecule	Mass calibration nmol/l	Molecular weight
hCG (CHO-cell recombinant)	0.53	36,700
Hyperglycosylated hCG (JEG-3 cell line)	5.4	40,500
Nicked hCG (batch M4)	9.1	36,700
Nicked hCG missing βCTP (batch M2B)	1.4	28,400
Nicked hyperglycosylated hCG (batch C5)	1.2	40,500
Asialo hCG (CHO-cell recombinant)	0.95	34,000
hCGβ (CHO-cell recombinant)	0.35	22,200
Nicked hCGβ (batch M4)	0.48	22,200
β-core fragment (batch P9)	0.20	10,300

C-terminal peptide was considered mandatory. For Down syndrome screening, both hCGβ and hyperglycosylated hCG are elevated and considered critical component in screening. Results are examined here for each platform total hCG assay alphabetically.

3.1. Architect total hCG

The Architect test appropriately detected hCG, hyperglycosylated hCG and hCGβ (Table 3). This test had 0% recognition of nicked hCG missing the C-terminal peptide, just 1% recognition of β-core fragment, and poorly detected nicked hCG, nicked hCGβ, asialo hCG and nicked hyperglycosylated hCG, or poor detection of 6 of 9 standards. This test has no significant LH cross-reactivity and seemingly detected urine samples as well as serum samples. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.2. AxSYM Total hCG

The AxSYM test was like the Architect, suggesting the use of similar antibodies in the 2 tests. The Axsym total hCG test detected hCG, hyperglycosylated hCG, nicked hCG and hCGβ appropriately (Table 3). This test had 0% recognition of hCG missing the C-terminal peptide, just 1% recognition of β-core fragment, and poorly detected nicked hCGβ, asialo hCG and nicked hyperglycosylated hCG, or poor detection of 5 of 9 standards. This test has no significant LH cross-reactivity and seemingly detected urine samples as well as serum samples. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.3. Access 2 total hCG

The Access 2 test detected hCG, hyperglycosylated hCG and nicked hCG appropriately (Table 3). This test gave a grossly exaggerated result with hCGβ. This test had 0% recognition of hCG missing the C-terminal peptide, just 1% recognition of β-core fragment, and poorly detected nicked hCGβ, asialo hCG and nicked hyperglycosylated hCG, or poor detection of 6 of 9 standards. This test has no significant LH cross-reactivity and seemingly did not detect urine samples appropriately so cannot be used as a urine test. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.4. DxI 800 Total hCG

The DxI800 test detected only hCG and hyperglycosylated hCG appropriately (Table 3). This test gave a grossly exaggerated result with hCGβ. This test had 0% recognition of hCG missing the C-terminal peptide, just 1% recognition of β-core fragment, and poorly detected poorly detected nicked hCGβ, nicked hCG, asialo hCG and nicked hyperglycosylated, or poor detection of 7 of 9 standards. This test has no significant LH cross-reactivity and seemingly did not detect urine samples appropriately so cannot be used as a urine test. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.5. Vitros ECiQ total hCG

The Vitros EciQ total hCG test appropriately detected hCG, nicked hCG, nicked hyperglycosylated hCG and asialo hCG (Table 3). This tests had 0% recognition of nicked hCG missing the C-terminal peptide, just 1% recognition of β-core fragment, and gave unacceptable results with hCGβ (47% reactivity), and poor results with hyperglycosylated hCG (important for early pregnancy detection) and nicked hCGβ (the most common circulating form in cancer cases), or poor detection of 5 of 7 standards. This test had minor LH cross-reactivity and seemingly did not detect urine samples appropriately so cannot be used as a urine test. It is inferred that this test is only appropriate for advanced pregnancy detection (Table 3).

3.6. Elecsys hCG + β

The Elecsys hCG + β test appropriately detected hCG, hyperglycosylated hCG, nicked hyperglycosylated hCG, and hCGβ (Table 3). This

Table 3
Detection of serum hCG-related molecules by different automated total hCG tests. βCTP is β-subunit C-terminal peptide, RIA is radioimmunoassay. Percentage values are the result in nmol/l expressed as a percentage of the actual concentration of the standard (Table 2). A poor detection, <75% or >125% of standard concentration is shown by bold underlined numbers. Under recommended use, 3 refers to poor test, poor recognition of hCGβ-related molecules, 2 to limited use test, limited recognition of hCGβ-related molecules, and 1 to good test, good recognition of hCG-related molecules.

	Architect	AxSYM	Access 2	DxI 800	Vitros ECiQ	Elecsys hCG + β	ACS180	Centaur	Dimension	Immulite 2000	Stratus	A1A	Manual hCGβ RIA
Serum standards													
hCG	96%	103%	103%	100%	112%	109%	105%	104%	96%	96%	92%	95%	99%
Hyperglycosylated hCG	86%	85%	120%	98%	**68%**	78%	102%	81%	**67%**	105%	**66%**	nd	92%
Nicked hCG	**70%**	99%	84%	**71%**	80%	**69%**	85%	**66%**	**65%**	115%	**8%**	nd	98%
Nicked hCG missing CTP	**0%**	**0%**	**0%**	**0%**	**0%**	**12%**	**0%**	**0%**	**10%**	109%	**28%**	**16%**	95%
Nicked hyperglycosylated hCG	**40%**	**46%**	**46%**	**51%**	80%	100%	**70%**	**40%**	80%	103%	88%	**70%**	**70%**
Asialo hCG	**35%**	**69%**	**48%**	**46%**	85%	**46%**	81%	**39%**	**65%**	114%	**73%**	**59%**	**126%**
hCGβ	87%	94%	**142%**	**136%**	**47%**	102%	**126%**	**47%**	**47%**	111%	**73%**	**66%**	86%
Nicked hCGβ	**33%**	**51%**	**56%**	**63%**	**19%**	**53%**	**72%**	**19%**	**41%**	107%	**70%**	**60%**	80%
β-core fragment	**1%**	**1%**	**1%**	**1%**	**1%**	**16%**	**0%**	**1%**	**1%**	**35%**	**1%**	**1%**	76%
Bold underline poor detection	6 of 9	5 of 9	6 of 9	7 of 9	5 of 9	5 of 9	5 of 9	7 of 9	7 of 9	1 of 9	7 of 9	6 of 7	2 of 9
Score, number of poor detections	2	2	2	3	2	2	2	3	3	1	3	3	1
LH cross-reactivity	0.01%	0.01%	0.01%	0.01%	0.32%	0.01%	0.01%	0.01%	0.01%	0.04%	0.53%	0.01%	0.36%
Urine standards													
Urine hCG	103%	118%	**69%**	85%	**58%**	100%	106%	108%	91%	107%	88%	103%	101%
Urine hCGβ	84%	80%	119%	111%	**11%**	86%	nd	**44%**	**35%**	120%	**70%**	**63%**	nd
Application (based on detection)													
Pregnancy (6 weeks–term)													
Early (3/4/5 weeks) pregnancy													
Abnormal pregnancy													
Down syndrome screening													
Non trophoblastic cancer													

tests had 12% recognition of nicked hCG missing the C-terminal peptide, 16% recognition of β-core fragment, and low results with nicked hCG, asialo hCG, and nicked hCGβ, or poor detection of 5 of 7 standards. This test seeming does not use a C-terminal peptide capture or tracer antibody allowing some detection of hCG minus C-terminal peptide and β-core fragment determinants. This test had minimal LH cross-reactivity and seemingly detected urine samples appropriately so can be used as a urine test. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.7. ACS180 total hCG

The ACS180 tests appropriately detected hCG, hyperglycosylated hCG, nicked hCG and asialo hCG (Table 3). This tests had 0% recognition of nicked hCG missing the C-terminal peptide, 0% recognition of β-core fragment, and unduly high results with hCGβ, and unduly poor reactivity with nicked hyperglycosylated hCG and nicked hCGβ, or poor detection of 5 of 9 standards. This test had minimal LH cross-reactivity and seemingly detected urine samples appropriately so can be used as a urine test. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.8. Centaur total hCG

The assay uses similar antibodies to those used in the ACS180 test, yet performed here much poorer than the ACS180. The Centaur tests appropriately detected only hCG and hyperglycosylated hCG (Table 3). This test had 0% recognition of nicked hCG missing the C-terminal peptide, just 1% recognition of β-core fragment, and unduly low results with hCGβ (47% recognition). This assay also did not appropriately detect the important nicked hCG, nicked hyperglycosylated hCG, asialo hCG or nicked hCGβ. Poor detection was noted with 7 of 9 standards. This test had minimal LH cross-reactivity and seemingly could detected urine samples appropriately so can be used as a urine test. It is inferred that this test is appropriate only for advanced pregnancy detection and early pregnancy detection (Table 3).

3.9. Dimension vista total hCG

The Dimension Vista assay appropriately detected only hCG and nicked hyperglycosylated hCG (Table 3). This test had 10% recognition of nicked hCG missing the C-terminal peptide, 1% recognition of β-core fragment, and a unduly low result with hCGβ (47% detection). This assay also did not appropriately detect hyperglycosylated hCG, the critical molecule of early pregnancy, nicked hCG, asialo hCG or nicked hCGβ. Poor detection was noted with 7 of 9 standards. This test had minimal LH cross-reactivity and seemingly could detect urine samples appropriately and can be used as a urine test. It is inferred that this test is only appropriate for advanced pregnancy detection (Table 3).

3.10. Immulite total hCG

As reported in previous studies of assay specificity [1,13] the Immulite test seeming appropriately detects most hCG related molecules and hCG variants. The Immulite 2000 test appropriately detected 8 of 9 hCG related molecules and hCG variants, making it very different to all the other automated assays. These included hCG, hyperglycosylated hCG, nicked hCG, nicked hCG minus the C-terminal peptide, nicked hyperglycosylated hCG, asialo hCG, hCGβ and nicked hCGβ (Table 3). The only test that the Immulite failed on was β-core fragment, 35% detection. This percentage is small, but is significantly better than the percentage achieved by all other automated tests. In terms of appropriate detection of hCG in normal and abnormal pregnancies and cancers (Table 1, Fig. 1–3) this test is exceptional compared to every other automated test, and is probably the only automated test that can really call itself a total hCG test, with very trustworthy clinical results. This test clearly outperforms all other automated test, it is appropriate for all hCG-related applications. This test had minimal LH cross-reactivity and seemingly could detected urine samples appropriately so can be used as a urine test (Table 3).

3.11. Stratus total hCG

The Stratus test appropriately detected only hCG and nicked hyperglycosylated hCG (Table 3). It inappropriately detected hyperglycosylated hCG, a critical molecule in pregnancy detection, hCGβ a second critical molecule, nicked hCG, nicked hCG missing the C-terminal peptide (28% detection), asialo hCG, nicked hCGβ and β-core fragment (1% detection). Most notably this assay failed to detect appropriately nicked hCG, an important serum determinant (8% detection). This test failed to detect 7 of 9 standards including critical standards such as hyperglycosylated hCG and nicked hCG. This test had a high cross-reactivity with LH (0.53%). If LH was high as seen in menopause, this test could show positive for hCG. This test was of no use in detecting urine hCG. It is inferred that this test is only appropriate for advanced pregnancy detection (Table 3).

3.12. A1A total hCG

The A1A test was only evaluated with 7 hCG determinants. Of the 7 determinants it only appropriately detected hCG itself. The A1A test failed to detect hCG reactivity appropriately in nicked hCG missing the C-terminal peptide (16% detection), nicked hyperglycosylated hCG, asialo hCG, critical hCGβ, nicked hCGβ and β-core fragment (1% detection) or in 6 of 7 tests. This test had minimal LH cross-reactivity and seemingly could detected urine samples appropriately so can be used as a urine test. It is inferred that this test is appropriate for advanced pregnancy detection, early pregnancy testing and Down syndrome screening (Table 3).

3.13. Manual hCGβ RIA total hCG

The hCGβ RIA detected all hCG related molecules and variants quite effectively. This explains why two specialist gestational trophoblastic disease groups, Charing Cross Trophoblast Center in London, The Netherland Gestational Trophoblastic Disease center, choose this classic assay in preference to an automated test. The hCGβ RIA appropriately detected hCG, hyperglycosylated hCG, nicked hCG, nicked hCG missing the C-terminal peptide (95% detection), hCGβ, nicked hCGβ and β-core fragment (76% detection). This tests over detected asialo hCG, and poorly detected nicked hyperglycosylated hCG. In total this test detected 7 of 9 hCG forms. In terms of appropriate detection of hCG in normal and abnormal pregnancies and cancers (Table 1, Figs. 1–3) this test is exceptional compared to most automated test, with very trustworthy results. The hCGβ RIA total hCG test can be used for all applications. This test does like no other test appropriately detect hCG missing the C-terminal peptide and β-core fragment. This test has low LH cross-reactivity and appropriately detect urine hCG (Table 3).

4. Comments

Sturgeon et al. [16] and Whittington et al. [23] also examined the specificity automated total hCG tests. The only standards available to them were the WHO standards. These standards are in many ways defective. Firstly, for a strange mixture of hCG derivatives. Not the common hCG variants in serum and urine. They have WHO standards for- hCG, nicked hCG, hCGβ, nicked hCGβ, hCG β-core fragment and

hCGα. While no commercial total hCG test detects hCGα, they miss out all the critical early pregnancy variants, gestational trophoblastic disease variants, and cancer variants. They have no standard for hyperglycosylated hCG or nicked hyperglycosylated hCG, the two principal molecules produced in early pregnancy and in trophoblastic malignancies. They also have no standards for nicked hCG missing the βC-terminal peptide or hCGβ missing the C-terminal peptide. Two critical molecules in detecting pregnancy post parturition or following pregnancy termination, or for testing cancer antigens. The other major problem with the WHO standard is that they are some ways quite crude. The hCG standard contains 10% nicked hCG and 10% hyperglycosylated hCG. The nicked hCG standard contains approximately 30% nicked hyperglycosylated hCG and 17.5% hCG, their hCGβ standard contains 20% nicked hCGβ.

Realizing in 1990 the problem with hCG standards, we prepared 30 urine pools from pregnancy women, women with hydatidiform mole and women with choriocarcinoma. We then purified the hCG, free β (hCGβ) and β-core fragment in the large pools using the chromatography and the Reverse Phase methods of Birken et al. [6,24]. We ended up with pure standards for- Nicked hCG and hCGβ (100% nicked), nicked hCG and hCGβ missing the βC-terminal peptide, hyperglycosylated hCG and nicked hyperglycosylated hCG (100% nicked). What was great is that we had the complete peptide and carbohydrate structure of all these purified preparations [6]. It is our belief that these standards give us a unique advantage.

Interestingly, Sturgeon et al. [16] found some strange β-core fragment results. As claimed, the Elecsys total hCG assays detected 35% β-core fragment, and the Ortho Vitros ECi assay detects 17.2% β-core fragment, while the Siemens Immulite 2000 assay detects 65% β-core fragment. All these values at the time of publication appeared to us to be strangely high results. We showed, as we have shown on previous occasions [1,14], that the Elecsys total hCG test actually detects 16% β-core fragment, that the Vitros Eci detects 1% and that the Immulite 2000 assays detects 35% β-core fragment. Our results suggest that the Sturgeon β-core fragment standard was 2 of more fold off in calibration.

An interesting saga was generated by the Sturgeon β-core fragment study [16]. In 2010, Stephen DuToit wrote to me from New Zealand. He wanted to adopt the Elecsys total hCG assay for cancer screening. As related it detects all hCG breakdown products including 35% β-core fragment. I wrote back to him, saying that this was wrong based on our 2004 study. He quoted the Sturgeon study [16]. I then sent to him in New Zealand our calibrated β-core fragment standard for him to test. True enough he calculated 16% detection and not 35% detection. I then invited Stephen DuTroit to join this study, testing our blinded standards in multiple assays in New Zealand.

Other problems were found with the Sturgeon findings [16]. They indicate that the Elecsys total hCG assay detects nicked hCG with 96.5% efficiency. We show in 2011 that it detects nicked hCG with a poor 69% efficiency. Which report is correct? To arbitrate, in 2004 we examined the Elecsys. We evaluated it at 3 independent laboratories. The results were 69%, 77% and 81%. The average result, 76% was presented. We believe that this confirms our result of 69% in 2011 is most likely correct. Similarly, in the study by Whittington et al. in 2009, they claim that nicked hCG was detected with 94.6% to 98.3% efficiency in 8 of 8 assays evaluated. Just like with the Elecsys detection of nicked hCG we cannot agree with this. On careful evaluation all 12 automated hCG assay detected nicked hCG with 8%, 65%, 66%, 69%, 70%, 71%, 80%, 84%, 84%, 98%, 99% and 115% efficiency. The standard for nicked hCG that we used was M4 [6]. M4 hCG was 100% nicked at β47–48, and not invariably nicked at β34–35, 36–37 and 38–39, and hyperglycosylated like WHO standards [6].

5. Summary

From the perspective of detection of hCG standards, all assays appropriately detected pure hCG, the principal molecule produced during advanced pregnancy (6 weeks gestation to term). Three assays (Vitros ECiQ, Dimension and Stratus) failed to appropriately detect hyperglycosylated hCG, the principal molecule produce in early pregnancy (Table 1, Fig. 3). Six assays (Architect, Dxl 800, Elecsys hCG + β, Centaur, Dimension, Stratus) failed to appropriately detect nicked hCG, a key molecule in third trimester pregnancy and clearing hCG following parturition or dilation and curettage (Table 1, Figs. 1 and 2). All automated assay except the Siemens Immulite failed to detect nicked hCG missing the C-terminal peptide, the terminal hCG degradation product in serum in cases of spontaneous abortion, ectopic pregnancy and gestational trophoblastic disease (Table 1, Figs. 1 and 2). Most automated tests, Architect, Axsym, Access 2, Dxl 800, Elecsys hCG + β, Centaur, Dimension, Stratus, A1A) failed to appropriately detect asialo hCG. Sialic acid is a major variable, patient to patient, in hCG [6,22]. It varies the greatest in failing pregnancies, and during the second and third trimesters of pregnancy. Surprisingly, most tests or eight of the 12 automated tests (Access 2, Dxl 800, Vitros ECiQ, ACS 180, Centaur, Dimension, Stratus, A1A) failed to appropriately detect hCGβ. If total hCG is broadly defines as hCG plus hCGβ, how do 8 of 12 tests label themselves at total hCG. hCGβ detection is important in early pregnancy, gestational trophoblastic diseases, and is critical to the detection of non-trophoblastic neoplasms (Table 1). In this testing, the Immulite stood out as the only series of test that detected all hCG standards and hCG degradation products standards. The only exception was detection of β-core fragment, 35% detection. β-core fragment is only present in urine samples, as the terminal degradation product of hCGβ. The Immulite is seemingly appropriate for all hCG test applications (advanced pregnancy detection, early pregnancy detection, abnormal pregnancy detection, Down syndrome screening, monitoring non-trophoblastic cancer).

From the perspective of hCG test applications, only the Immulite series of tests and the classic hCGβ RIA test were considered satisfactory for all hCG applications. A total of 7 of 12 automated assay types were considered limited in application, and useful for advanced pregnancy detection, early pregnancy detection and Down syndrome screening. These tests were mostly limited by not detecting molecules missing the C-terminal peptide. One of 12 tests was found only appropriate for advanced pregnancy detection and early pregnancy detection. This test was limited by non-detection of molecules missing the C-terminal peptide and by failure to appropriately detect hCGβ. Finally, 3 tests were found to be limited in use, with the only application being advanced pregnancy. These tests were not appropriate for early pregnancy detection because of poor recognition of hyperglycosylated hCG. Manufacturers of these tests need to urgently fix the hyperglycosylated hCG detection problem.

Have hCG tests improved since the 1970s and the time of the hCGβ RIA. We say no they have not. Yes, more rapid and automated tests have been developed, but in terms of specificity for appropriately detecting hCG and its variants, the answer is clearly no. One line of automated tests, the Siemens Immulite test, does maintain appropriate wide specificity, detecting appropriately nicked hCG missing the C-terminal peptide, and partly detecting urine β-core fragment. While most manufactures design tests as using an hCGβ-subunit core antibody as capture antibody, and a β-C-terminal peptide antibody as tracer, or the reverse, this is not a clever configuration in that it prevents detection of nicked hCG missing the C-terminal peptide and β-core fragment. The Immulite series uses a different combination of antibodies, giving their assay wide specificity. This is two antibodies, capture and tracer, to two sites in hCGβ-subunit core.

Why is detection of urine hCG, particularly β-core fragment important? When hCG clears the circulation, such as at parturition, following ectopic pregnancy and spontaneous abortion, at parturition, and in following gestational trophoblastic diseases, it clears the urine significantly slower than it clears the circulation (about 3–4 days longer). Many times, the USA hCG Reference Service has detected residual hCG in the urine after no hCG being detected in serum.

Detection of total hCG in urine is invaluable in evaluating a potential case of questionable false positive hCG, or a case that is positive in an hCG test and is not pregnant. All false positive hCG cases are negative in the urine and positive only in the serum [21]. FDA regulations state that urine may only be evaluated in an insensitive quantitative manner. We claim that if you use the Architect, AxSym, Elecsys hCG + β, ACS180, Centaur, Dimension, Immulite or A1A total hCG assay that you can freely evaluate urine samples in clearance samples or for false positive testing, but only claim them on the report as research values.

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