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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 DxI 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

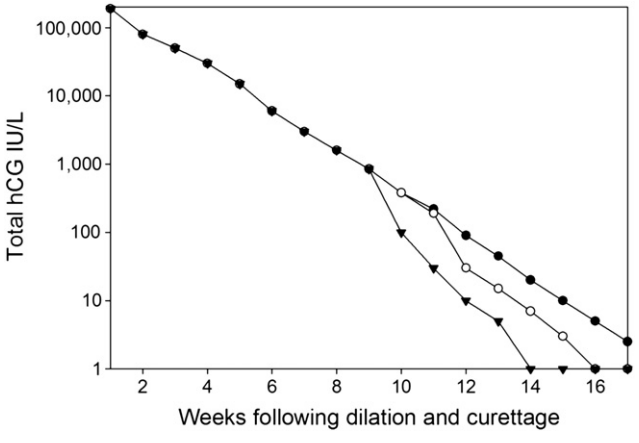

Fig. 1. Example of limitation of assay poorly detecting nicked hCG and nicked hCG missing the β-C-terminal peptide, following weekly the dilation and curettage of a complete hydatidiform mole. Black circles are the Siemens Immulite 1000 total hCG assay. Open circles are the AxSym total hCG assay (does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

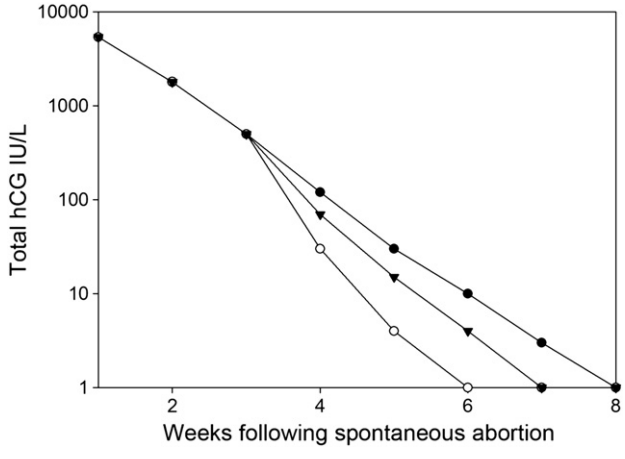

Fig. 2. Example of limitation of assay poorly detecting nicked hCG and nicked hCG missing the β-C-terminal peptide, following weekly the spontaneous abortion of pregnancy in the first trimester. Black circles are the Siemens Immulite 1000 assay. Open circles are the AxSym total hCG assay (does not appropriately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