

# Selecting an Appropriate hCG Test for Managing Gestational Trophoblastic Disease and Cancer

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Human chorionic gonadotropin (hCG) is a glycoprotein composed of 2 dissimilar subunits,  $\alpha$  and  $\beta$ , joined non-covalently. This hormone is not only heterogeneous in peptide structure but also in combination of subunits and in the structure of carbohydrate side chains. Common hCG-related molecules in serum samples include regular hCG, hyperglycosylated hCG (ITA), nicked hCG, nicked ITA, hCG missing the

$\beta$ -subunit C-terminal extension, free  $\alpha$ -subunit, free  $\beta$ -subunit, free  $\beta$ -subunit missing the C-terminal extension, hyperglycosylated free  $\beta$ -subunit and nicked free  $\beta$ -subunit. The same molecules plus  $\beta$ -core fragment are present in urine samples. While ITA and regular hCG predominate in pregnancy samples, any one of these multiple hCG-related molecules may be the principal source of immunoreactivity in gestational trophoblastic disease, gestational trophoblastic neoplasm, choriocarcinoma and placental site tumor cases as well as in testicular cancer and germ cell tumor. As such it is critical to appropriately detect all these isoforms in the management of these diseases. Only 2 tests, the DPC Immulite (DPC, Inc., Los Angeles, California) and U.K. RIA (radioimmunoassay) (used at Charing Cross Hospital, London) appropriately detect all these hCG-related molecules. False positive hCG results are a major problem in the management of gestational trophoblastic disease and cancer. A partic-

ular problem is observed with the Abbott AxSym test. This test is flawed in design. It should be avoided in the management of gestational trophoblastic disease and

cancer. As shown in a blind study, a proportion of false positive samples in the Abbott AxSym test (Abbott Laboratories, Inc., Chicago, Illinois) can also be false positive with the U.K. RIA; none are false positive with the DPC Immulite test. Re-

## False positive hCG results are a major problem in the management of gestational trophoblastic disease and cancer today.

sults clearly show that the DPC Immulite/Immulite 2000 is the only appropriate assay for monitoring patients with gestational trophoblastic disease or cancer. (J Reprod Med 2004;49:545–553)

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Human chorionic gonadotropin (hCG) is a glycoprotein composed of 2 dissimilar subunits, an  $\alpha$ - and  $\beta$ -subunit, held together by charge interactions. hCG is an unusual glycoprotein in that as little as 65% of the molecular weight (MW) is due to amino acids or protein. The balance is due to large sugar side chains. hCG is sometimes considered a mucopolysaccharide, like collagen, because of the large carbohydrate component. There are 4 asparagine-

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linked (N-linked) sugar side chains with 9–15 sugar residues attached to each structure on hCG, 2 on the  $\alpha$ -subunit and 2 on the  $\beta$ -subunit. In addition, there are 4 serine-linked (O-linked) sugar side chains with 3–6 sugar residues on each structure, all on the C-terminal extension of the  $\beta$ -subunit (residues 92–145).

The combination of 2 subunits and 8 sugar side chains results in major variability in hCG structure. In addition to regular hCG (hCG), at least 5 general categories of variants are present on serum samples: hyperglycosylated hCG (invasive trophoblast antigen [ITA]), nicked hCG, hCG missing the  $\beta$ -subunit C-terminal extension, free  $\beta$ -subunit and nicked free  $\beta$ -subunit, and multiple combinations of these variations (e.g., nicked hyperglycosylated hCG missing the  $\beta$ -subunit C-terminal extension), and carbohydrate and sialic acid content variants.<sup>1–9</sup> The same 5 general categories of variants plus  $\beta$ -core fragment and  $\beta$ -core fragment carbohydrate variants are detected in urine samples.<sup>1–9</sup> Table I summarizes the structure of the key categories of hCG-related molecules, which vary in size from  $\beta$ -core fragment (MW ~10,000) to ITA (MW ~40,000).

Over 40 different commercial professional laboratory hCG tests are available in the United States for quantifying serum and urine hCG levels.<sup>4,10</sup> Most of these tests work through the multiantibody “immunometric assay” method. These tests use multiple antibodies against different sites on hCG and hCG-related molecules. An antibody to 1 or 2 sites is immobilized in a vial or attached to magnetic particles or microparticles. This antibody binds and immobilizes the hCG and hCG-related molecules. A further antibody is conjugated to a tracer. This tracer is either a dye, an enzyme that reacts with a substrate to form a spectrometrically or fluorimetrically detected color, or a radioactive or chemiluminescent agent. This tracer antibody binds the immobilized hCG and related molecules at an alternative site. This forms an immobilized complex, or “hCG sandwich”: tracer antibody  $\rightarrow$  hCG  $\rightarrow$  immobilized antibody. After washing, the tracer or color formed is measured by either spectrometry, fluorimetry, chemiluminescence or radioactivity. The amount of tracer is directly proportional to the total concentration of hCG and hCG  $\beta$ -subunit-related molecules present or total immunoreactivity.

**Table I** Structure of General Categories of hCG-Related Molecules Detected, to Different Extents, by Commercial hCG Immunoassays in Serum and Urine Samples in Pregnancy and Trophoblastic Disease

hCG-related molecule	Structure
Regular hCG (MW 36,000)	$\alpha$ -Subunit: 92 amino acids, no cleavages Mono- (8 sugars) and bi- (11 sugars) antennary N-linked side chains $\beta$ -Subunit: 145 amino acids, no cleavages Biantennary $\pm$ fucose (11 or 12 sugars) N-linked and mostly trisaccharide (3 sugars) O-linked sugar side chains
Hyperglycosylated hCG (MW 40,000)	$\alpha$ -Subunit: 92 amino acids, no cleavages Mono- (8 sugars) and biantennary + fucose (12 sugars) N-linked side chains $\beta$ -Subunit: 145 amino acids, no cleavages Bi- (11 or 12 sugars) and triantennary $\pm$ fucose (14 or 15 sugars) N-linked and hexasaccharide O-linked sugar side chains
Nicked hCG <sup>1</sup> (MW 36,000)	$\alpha$ -Subunit: 92 amino acids, no cleavages Mono- (8 sugars) and bi- (11 sugars) antennary N-linked side chains $\beta$ -Subunit: 145 amino acids, cleaved at $\beta$ 47–48, $\beta$ 43–44 or $\beta$ 44–45 Biantennary $\pm$ fucose (11 or 12 sugars) N-linked and mostly trisaccharide (3 sugars) O-linked sugar side chains
hCG missing $\beta$ -subunit C-terminal extension <sup>1</sup> (MW 29,000)	$\alpha$ -Subunit: 92 amino acids, no cleavages Mono- (8 sugars) and bi- (11 sugars) antennary N-linked side chains $\beta$ -Subunit: residues 1–92, C-terminal extension absent Biantennary $\pm$ fucose (11 or 12 sugars) N-linked and mostly trisaccharide (3 sugars) O-linked sugar side chains
Free $\beta$ -subunit <sup>1</sup> (MW 22,000)	No $\alpha$ -subunit; $\beta$ -subunit: 145 amino acids, no cleavages Biantennary $\pm$ fucose (11 or 12 sugars) N-linked and mostly trisaccharide (3 sugars) O-linked sugar side chains
Nicked free $\beta$ -subunit <sup>1</sup> (MW 22,000)	No $\alpha$ -subunit; $\beta$ -subunit: 145 amino acids, cleaved at $\beta$ 47–48, $\beta$ 43–44 or $\beta$ 44–45 Biantennary $\pm$ fucose (11 or 12 sugars) N-linked and mostly trisaccharide (3 sugars) O-linked sugar side chains
Urine $\beta$ -core fragment (MW 10,000)	No $\alpha$ -subunit; $\beta$ -subunit: 2 peptides $\beta$ -subunit residues 6–40 linked to 55–92 Degraded biantennary (3–5 sugars) N-linked and no O-linked side chains

Each category includes carbohydrate variants, especially sialic acid and charge variants.<sup>1–9</sup>

<sup>1</sup>Combinations of hCG variants are present in serum and urine: hyperglycosylated nicked hCG, nicked hCG missing  $\beta$ -subunit C-terminal extension, hyperglycosylated free  $\beta$ -subunit, nicked-hyperglycosylated free  $\beta$ -subunit and nicked free  $\beta$ -subunit missing the C-terminal extension and others.

A small number of laboratories, especially in Europe and Asia, continue to use the old and time-consuming competitive radioimmunoassay (RIA) methods, in which an unknown concentration of the antigen competes in binding radioactive antigen for binding a limited amount of antisera. An inverse relationship exists between the amount of radioactivity bound and the amount of unknown antigen band. All tests, whether RIA or immunometric assay, use at least 1 antibody directed against the  $\beta$ -subunit to differentiate hCG and luteinizing hormone; thus, the term  *$\beta$ -hCG test* commonly refers to any test detecting hCG or its  $\beta$ -subunit. For the purposes of this paper an hCG and  $\beta$ -hCG test are the same.

Professional laboratory hCG tests use antibodies to different sites on the  $\beta$ -subunit together with antibodies directed to an alternate site on the  $\beta$ -subunit,  $\beta$ -subunit C-terminal extension, hCG dimer, subunit interface, free subunits or  $\alpha$ -subunit. Because of these variations in antibody use, different commercial hCG tests may measure very different combinations of hCG-related molecules. Some tests detect regular hCG only; others detect all 7 major hCG-related molecules.<sup>4,6,8-11</sup> This may not be a problem for testing and monitoring normal pregnancies because regular hCG dominates in serum samples from 6 weeks of gestation until term. It is, however, a major concern in monitoring patients with hydatidiform mole. In these cases, nicked hCG, free  $\beta$ -subunit or nicked hCG missing the  $\beta$ -subunit C-terminal extension may at some times during management be the principal form of hCG present. It may also be a problem in cases of gestational trophoblastic neoplasm, choriocarcinoma, placental site trophoblastic tumor or germ cell tumors, in which ITA is commonly the principal form of hCG present.<sup>4,8,11</sup> Furthermore, since urine is commonly measured in gestational trophoblastic disease,<sup>14-16</sup> as a long-term follow-up and confirmatory medium, it is important to detect  $\beta$ -core fragment, the principal source of  $\beta$ -hCG immunoreactivity in urine.

Failure to appropriately detect all these hCG variants is a common cause of failure to detect active disease, or recurrence or persistence of trophoblastic disease.<sup>4-6,9</sup> This article considers all aspects of hCG detection in gestational trophoblastic disease. It advises on the use of hCG tests to ensure that the laboratory one is using employs an appropriate hCG test for managing gestational trophoblastic disease, testicular malignancies and germ cell tu-

mors. It also examines the problem of false positive hCG results and how to avoid false results. Problems identified with specific hCG tests are reported.

### ***Assay of hCG and hCG-Related Molecules in Gestational Trophoblastic Disease***

Recent studies have shown that ITA is the predominant form of hCG produced in the 3 weeks following implantation of pregnancy.<sup>1,3</sup> During the first, second, third and fourth weeks after implantation, ITA is gradually replaced with hCG during this period, accounting for >80%, 63% and 50%, respectively, of total hCG forms.<sup>1-3</sup> ITA is produced by invasive cytotrophoblast cells in early pregnancy.<sup>3,12</sup> ITA is also the principal hCG-related molecule produced by invasive cytotrophoblast cells in choriocarcinoma as well as in testicular and other germ cell malignancies.<sup>8,10,12</sup> In an initial blind study in 2001 using blind tests run at laboratories in the United States it was shown that assays invariably detect ITA (Table I). In a further blind study in 2003 involving laboratories in Canada, the United States and the United Kingdom, 11 commercial hCG assays were evaluated with ITA standards (924 mIU/mL) (Table II). Results varied greatly, from 1,544 to 468 mIU/mL (Table II) and from 167% to 51% of the standard. Table II shows that some tests, like the Dade Dimension RXL (Dade Behring, Inc., Deerfield, Illinois) and Beckman Access/Access-2 (Beckman Coulter, Inc., Fullerton, California), 2 of the principal tests used in the United States (Table III), and the Roche Elecsys E170 yield the most exaggerated or underexaggerated results and probably inappropriate and inaccurate ITA measurements for monitoring choriocarcinoma and testicular and other germ cell malignancies. The closest results to the calibrated standards were observed with the UK RIA (Charing Cross Hospital, London, U.K.), Tosoh A1A600 (Tosoh Bioscience, San Francisco, California), DPC Immulite/Immulite 2000 (DPC, Inc., Los Angeles, California) and Bayer ACS180 (Bayer Diagnostics, Pittsburgh, Pennsylvania) tests (Table II). It clearly is important to use a test that appropriately detects ITA for management of invasive disease. As described in another article in this symposium, the specific measurement of ITA may be useful in differentiating invasive and noninvasive gestational trophoblastic disease.

As shown previously,<sup>5,6</sup> circulating hCG (from hydatidiform mole) or ITA (from invasive disease) becomes cleaved or nicked as concentrations of

**Table II** Ability of Common Brands of Professional Laboratory Serum hCG Assays to Measure hCG Metabolic Products Commonly Found in Individuals with Gestational Trophoblastic Diseases

Standard	DPC Immulite/ 2000	Beckman Access/ Access 2	Abbott AxSym hCG	Dade Stratus Intact hCG	Bayer ACS180	Bayer ADVIA Centaur	Serono MAIAclone	USA RIA*
hCG free $\beta$ -subunit	✓	✓	✓	XX	X	X	XX	✓
ITA	✓	✓	✓	✓	✓	✓	X	✓
Nicked hCG	✓	✓	✓	✓	✓	✓	XX	✓
hCG minus C-terminal extension	✓	XX	XX	✓	XX	XX	X	✓
Urine $\beta$ -core fragment	✓	XX	XX	XX	XX	XX	XX	✓
No. of low results (<150 mIU/mL)	0 of 5	2 of 5	2 of 5	2 of 5	2 of 4	2 of 4	4 of 5	0 of 5

All standards were calibrated by amino acid analysis and converted into international units based upon MW and molar equivalents of hCG. The concentration of all standards was 200 mIU/mL. Samples were coded and tested blindly. Antigens weakly detected in a specific assay (result <150 mIU/mL or <75% of calibrated concentration) are indicated with an X, and those extremely weakly detected or not detected at all (<50 mIU/mL or <25% of calibrated concentration) are indicated as XX.

In all cases the unit of measure is mIU/mL.

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hCG and hCG-related molecules diminish after evacuation or chemotherapy or while hCG results are carefully monitored as they become undetectable. hCG and ITA are nicked or cleaved between residues  $\beta$ 43-44,  $\beta$ 44-45 or  $\beta$ 47-48 in the middle of the molecule. This nicking occurs at 1 of the principal  $\alpha$ -subunit/ $\beta$ -subunit interaction sites.<sup>7</sup> As such, nicking leads to rapid dissociation of hCG molecules, releasing a nicked free  $\beta$ -subunit.<sup>8</sup> In trophoblast disease cases and other hCG-producing tumors (testicular cancer and other germ cell malignancies), when hCG results fall below 100 mIU/mL, the combination of nicked hCG and its dissociation product, nicked free  $\beta$ -subunit, commonly becomes the major or sole sources of hCG immunoreactivity in serum and urine.<sup>5,6,9</sup> We have observed cases in which a recurrence of invasive disease or persistence of a hydatidiform mole has

been completely missed by use of an assay that does not appropriately detect nicked hCG or free  $\beta$ -subunit.<sup>5</sup> It appears that measurement of these molecules is essential for accurately monitoring hCG levels until they become undetectable or reach background (<2 mIU/mL) concentrations.<sup>5,6,9</sup> It is also essential for demonstrating that background hCG and related-molecule concentrations remain undetectable for appropriately detecting persistence or recurrence of disease. As shown in a 2001 report (Table I) and 2003 report (Table II), assays invariably detect both nicked hCG and free  $\beta$ -subunit. Clearly, as shown in the tables, the Roche Elecsys E170 (Roche Diagnostics, Inc., Indianapolis, Indiana) and Serono MAIAclone (Serono International S.A., Geneva, Switzerland) assays ineffectively detected or failed to detect nicked hCG. While the Beckman Access/Access-2 significantly exaggerat-

**Table III** Detection of Free  $\beta$ -Subunit Standard, Nicked hCG and  $\beta$ -Core Fragment Standard by 11 Commercial hCG Tests

hCG Variant	Abbott AxSym	Bayer ACS- 180	Bayer ADVIA Centaur	Beckman Access/ Access 2	Dade	Dade Stratus $\beta$ -hCG	DPC Immulite/ 2000	Roche Elecsys E170	Tosoh AIA600	Ortho Vitros ECi
					Dimension Intact hCG					
ITA <sup>1</sup>	87	102	108	130	51	85	99	167	97	80
$\beta$ -hCG <sup>2</sup>	84	134	142	182	84	96	98	82	82	114
Nicked hCG <sup>3</sup>	112	85	84	107	101	102	117	75	105	77
hCG $\beta$ -core fragment <sup>4</sup>	ND	ND	ND	ND	ND	ND	81	ND	ND	ND

Standards were calibrated by amino acid analysis. Mass values were converted to hCG equivalents (mIU/mL) using the MWs and World Health Organization conversion factor, as published previously.<sup>4</sup> Values are percentage of standard concentration.

<sup>1</sup>Assays were tested with purified ITA, 84  $\mu$ g/L (equivalent of 924 mIU/mL); mean result for all 10 assays was 899 mIU/mL (97% of standard).

<sup>2</sup>Assays were tested with pure  $\beta$ -hCG, 230  $\mu$ g/L (molar equivalent of 3,910 mIU/mL); mean result for all 10 assays was 3,940 mIU/mL (100% of standard).

<sup>3</sup>Assays were tested with pure nicked hCG, 330  $\mu$ g/L (equivalent of 3,630 mIU/mL); mean result for all 10 assays was 3,463 mIU/mL (95% of standard).

<sup>4</sup>Assays were tested with pure hCG  $\beta$ -core fragment, 10.0  $\mu$ g/L (molar equivalent of 400 mIU/mL); mean result for the 2 assays detecting this antigen was 337 mIU/mL (84% of standard).

ND = not detected.

ed free  $\beta$ -subunit results, intact hCG tests, like the Dade Stratus Intact hCG (Dade Behring), failed to detect free  $\beta$ -subunit. Considering together nicked hCG and free  $\beta$ -subunit, its dissociation product, the closest results to the standards were observed with the Dade Stratus (102% and 96%, respectively), DPC Immulite/Immulite 2000 (98% and 117%, respectively) and UK RIA (92% and 96%, respectively).

In 2 independent studies we observed hCG missing the  $\beta$ -subunit C-terminal extension in serum from patients' with trophoblast disease.<sup>4,9</sup> In the first study we evaluated 10 serum samples from women with complete hydatidiform mole. In 1 case the hCG result was 3,500 mIU/mL, while it was 3,800 mIU/mL in 2 tests requiring the  $\beta$ -subunit C-terminal extension to be present. The same sample gave results of 77,000–201,000 mIU/mL in 8 other immunoassays.<sup>9</sup> Clearly, assays requiring the  $\beta$ -subunit C-terminal extension can give misleading results. In a more recent study, 5 of 76 cases of complete mole and choriocarcinoma, or about 1 in 15 cases, were shown to have multifold lower hCG results in 3 of 9 assays that used an antibody to the  $\beta$ -subunit C-terminal extension.<sup>4</sup> Clearly, detection of hCG missing the  $\beta$ -subunit C-terminal extension is critical for appropriate monitoring of gestational trophoblastic disease. Using an assay that detects the  $\beta$ -subunit C-terminal extension is the safest and most appropriate way to manage gestational trophoblastic disease. As shown by a 2001 study (Table I), only the DPC Immulite/Immulite 2000, Dade Stratus and UK RIA detect hCG missing the  $\beta$ -subunit C-terminal extension. Considering all these studies, the detection of hCG missing the  $\beta$ -subunit C-terminal extension and tests detecting this element, only the DPC Immulite/Immulite 2000 and UK RIA appropriately measure the key antigens, ITA, free  $\beta$ -subunit and nicked hCG. These 2 assays are clearly the best choice, when it comes to hCG assay specificity, for management of gestational trophoblastic disease and cancer. In support of the conclusion that the DPC Immulite/Immulite 2000 and UK RIA are the most appropriate tests is the observation that these are the only 2 (plus USA RIA) that detect  $\beta$ -core fragment, the principal immunoreactivity in urine samples, and all forms of hCG ranging from the largest molecule, ITA (MW ~ 40,000), to the smallest,  $\beta$ -core fragment (MW ~ 10,000).

Urine hCG measurements are very useful in the management and diagnosis of trophoblastic dis-

ease. Patients can readily collect and ship samples to clinical laboratories for long-term monitoring of trophoblastic disease. Such a system is used by the Trophoblast Disease Center at Charing Cross Hospital.<sup>14,15</sup> As discussed below, some women are erroneously treated for gestational trophoblastic disease because of false positive serum hCG results.<sup>4,11,17-31</sup> The interfering substance that causes false positive results, human heterophilic antibodies, is present in serum and not urine.<sup>4,10,12</sup> Publications from our laboratory<sup>10,11</sup> and literature provided by some hCG test manufacturers recommend the use of urine tests to confirm the validity of serum hCG results. As such, urine hCG measurements can also play a clear role in confirming hCG results. In most cases, urine  $\beta$ -core fragment is the principal hCG immunoreactive molecule in trophoblastic disease, testicular cancer and germ cell tumor patient urine. Use of a urine test that detects  $\beta$ -core fragment, like the DPC Immulite/Immulite 2000 or RIA, is important for urine measurement in gestational trophoblastic disease and cancer detection.

hCG-related molecules vary greatly in structure and size. hCG dissociation and breakdown products may be the sole source of hCG in the serum of patients with gestational trophoblastic disease and other hCG-producing malignancies (testicular cancer and germ cell tumors). It is important to talk as soon as possible with one's laboratory manager and make sure that he or she is using an appropriate test that detects all the pertinent hCG-related molecules and to consider outside options (i.e., a reference laboratory) before submitting samples for monitoring patients and drawing meaningful conclusions from the results.

### **False Positive hCG Results**

The USA hCG Reference Service, a consulting service specializing in aiding physicians in the interpretation of confusing hCG results, has identified 61 false positive cases, 10 with a history of gestational trophoblastic disease and 51 with none. Forty-eight of these 61 cases received needless therapy for gestational trophoblastic neoplasms because of the false positive results. In many cases therapy included cytotoxic combination chemotherapy, hysterectomy and other chemotherapy, surgery and patient-harming protocols.

Now, after nearly 5 years of operation and multiple publications on the false positive hCG problem,<sup>4,11,17-31</sup> we continue to observe a continuing

number of false positive hCG results. We estimate that in addition to the 61 cases identified at the USA hCG Reference Service, we have helped identify approximately 150 additional cases over the phone, with instructions to test urine and to repeat serum tests in an alternative assay. False positive results were identified according to the following criteria<sup>18-21</sup>:

1. The finding of >5-fold difference in serum hCG results with alternative immunoassays (critical criterion).
2. The presence of hCG in serum and absence of detectable hCG or hCG-related molecule immunoreactivity in a parallel urine sample (critical criterion).
3. The observation of false positive results in other tests for molecules not normally present in serum, such as urine  $\beta$ -core fragment (confirmatory criterion)
4. The finding that a heterophilic antibody-blocking agent (Scantibodies Inc. HBR, La Jolla, California) prevented or limited false detection (confirmatory criterion).

Other laboratories have also identified patients undergoing needless therapy due to false positive hCG results in modern tests.<sup>24-26,29,31</sup> False positive hCG results were also reported in the 1980s using the older RIA technology.<sup>22,23</sup> It was thought then with the change to modern assays, that false positive hCG due to interfering heterophilic antibodies was an old problem associated with the RIA and would disappear with replacement of the old technology.<sup>32</sup> This is clearly not what has happened.<sup>17-21,24-31</sup> A false positive hCG result arises from a specific commercial laboratory hCG test used at the medical center's clinical laboratory. In most cases it is due to interfering heterophilic antibodies in the serum of antianimal IgG antibodies that replace hCG in the "hCG sandwich": tracer antibody  $\rightarrow$  antianimal IgG antibody  $\rightarrow$  immobilized antibody. One of the principal methods of identifying a false positive result is showing that other assays give very different or negative results. As such, the choice of hCG test used may make a difference in obtaining false positive results.

Table III lists the 8 most commonly used commercial laboratory hCG tests in the United States and Canada. As shown in Table III, of the 61 cases identified by the USA hCG Reference Service with false positive hCG, the vast majority of cases, 49 cases (80%), occurred with a single assay, the Abbott AxSym (used by 28% of laboratories). Further-

more, the Abbott AxSym consistently gave the highest concentration for the samples with false positive results. (The median result with the Abbott AxSym was 111 mIU/mL as compared with 7-53 mIU/mL in all other assays that at some point gave false positive results.) The Bayer ADVIA Centaur (Bayer Diagnostics) gave false positive results for 4 of the 61 cases; the Dade Dimension, Bayer ACS180 and Ortho Vitros Eci (Ortho Diagnostics Inc., Rochester, New York) each gave false positive results for 2 of the 61 cases; and the Tosoh Nexia and Bayer Immuno-1 each gave false positive results for 1 case. Three assays, among the 8 most commonly used assays in Canada and the United States have no record of false positive results. These are the Beckman Access, used by 13%; Roche Elecsys, used by 4%; and DPC Immulite, used by 2% of laboratories.<sup>33</sup>

False positive results following evacuation of hydatidiform mole or in cases with no history of gestational trophoblastic disease is a major problem today in management, leading to erroneous diagnosis and needless therapy. None of the false positive cases were from centers using the RIA. However, we know of only 1 commercial clinical laboratory each in the United States and U.K. using the RIA as compared with >2,300 laboratories in the United States using immunometric tests.<sup>33</sup>

As shown above, the DPC Immulite/Immulite 2000 and UK RIA are clearly the optimal choices for management of gestational trophoblastic disease and cancer. We found additional serum in our freezer from 11 cases, all of which had given false positive results with the Abbott AxSym. In all these cases false positive results were shown to be due to heterophilic antibodies.<sup>17-21</sup> We blindly evaluated these samples, together with 20 other true positive gestational trophoblastic disease samples, with the DPC Immulite and with the UK RIA at Charing Cross Hospital. As shown in Table IV, none of the 11 were positive with the DPC Immulite (<1 mIU/mL), but 3 of 11 were positive with the UK RIA. We also blindly tested the corresponding patient urines, naturally free of interfering heterophilic antibodies. While none of the 11 were positive with the DPC Immulite (<1 mIU/mL), 8 of 11 were positive in urine with the UK RIA (3-85 mIU/mL). This indicates a potential false positive detection problem in serum samples using the UK RIA, failure to recognize false positive results by showing absence in urine or a bizarre RIA false positive urine hCG problem.

**Table IV** False Positive Results in hCG Assays: The USA hCG Reference Service Reference Service Experience

hCG tests giving false positive results	% Of market share	No. of cases
Abbott AxSym	28	49, Mean false positive 111 mIU/mL
Dade Dimension RXT	26	2, Mean false positive 53 mIU/mL
Beckman Access/Access 2	13	0
Bayer ADVIA Centaur	6	4, Mean false positive 19 mIU/mL
Ortho Vitros ECI	5	2, Mean false positive 48 mIU/mL
Bayer ACS180	4	2, Mean false positive 39 mIU/mL
Rochce Elecsys E170	4	0
DPC Immulite/Immolute 2000	2	0

From the Reference Service experience with 61 cases, this table shows the occurrence of false positive hCG tests among the 8 most commonly used tests in the United States.<sup>33</sup>

This is a particularly worrisome situation. A false positive problem with RIA technology is very well established in the literature and was always a clear limitation of this technology.<sup>22,23,32</sup> This is a problem with the UK RIA. Charing Cross hospital has recently reported some worrisome results. Mitchell tested a large number of serum samples and identified 8 that were positive in the UK RIA (5–20 mIU/L) but gave negative results with the DPC Immulite test.<sup>14</sup> As described above, both tests recognize all forms of hCG present in serum and urine—the full range, from ITA (MW ~40,000) to  $\beta$ -core fragment (MW ~10,000), and the numerous forms between these extremes. Considering this situation, we question how the UK RIA can be detecting hCG forms not measurable by the DPC Immulite. The likely explanation, considering the above false positive problems with the UK RIA, is that these samples were false positive with the UK RIA not detected with the DPC Immulite. The conclusion is confirmed by an observation by Newlands et al<sup>15</sup> showing that in 12 patients with persistent elevated hCG in serum (UK RIA), only 2 had hCG in urine. This finding is seemingly consistent with false positive hCG. Clearly, considering false positive problems, the DPC assay is the test of choice.

#### **Clinical Chemistry of False Positive hCG: Flawed Test, Easy Fix**

The Abbott AxSym is used by 28% of laboratories in the United States and Canada and, it appears, by a much higher proportion of laboratories in Mexico, Australia and much of western Europe. Why does this single assay, which accounts for 28% of use in the United States, cause 80% of false positive cases and give by far the highest concentration in false positive cases?

The Abbott AxSYM total fl-hCG test reagent

manual recommends no dilution for serum samples up to 1,000 mIU/mL. The manual describes in detail the 3 components in the reagent pack: (1) a monoclonal anti- $\beta$ -hCG coated on microparticles in Tris buffer with protein stabilizers and azide preservative, (2) purified goat anti- $\beta$ -hCG conjugated to alkaline phosphatase in the same buffer/preservative, and (3) a sample diluent containing bovine and goat serum and azide preservative. The bovine and goat serum, or excess animal immunoglobulin, protects the purified goat anti- $\beta$ -hCG and the monoclonal antibody from heterophilic antibody interference. In this assay, this component is present only in the diluent. Undiluted samples would have no (or very minimal) animal serum or protection against heterophilic antibody interference (false positive results). As described above, all false positive results were <1,000 mIU/mL. In all likelihood these samples were all tested undiluted.

In 1 false positive case, we were provided with serial dilution data for the Abbott AxSYM total  $\beta$ -hCG test. When the serum was tested undiluted, the result was 600 mIU/mL; this was reported to the physician. At just 2-fold dilution (goat serum present), the result was <5 mIU/mL. Clearly, the addition of diluent containing goat serum completely suppressed the false positive result. We consider the absence/shortage of goat serum in undiluted samples to be the cause of the false positive results.

Ten serum samples were available from patients having false positive results with the Abbott AxSYM total  $\beta$ -hCG test. These were tested blindly by an outside laboratory (S.E.D. Medical Laboratories, Albuquerque, New Mexico) running the Abbott AxSYM test both undiluted and at just 2-fold dilution. Information on 7 other cases tested the

**Table V** *Blind Study of 11 Serum Samples Proven to Give False Positive Results with the Abbott AxSym hCG Test Due to Heterophilic Antibody Interference, Tested with the DPC Immulite and UK RIA*

Case no.	Result (mIU/mL)	
	DPC Immulite	UK RIA
1	<1 (<1)	7 (<2)
2	<1 (<1)	3 (6)
3	<1 (<1)	3 (6)
4	<1 (<1)	<2 (<2)
5	<1 (<1)	<2 (85)
6	<1 (<1)	<2 (11)
7	<1 (<1)	<2 (10)
8	<1 (<1)	<2 (10)
9	<1 (<1)	<2 (9)
10	<1 (<1)	<2 (11)
11	<1 (<1)	<2 (3)

Serum results are shown. Parallel urine sample results appear in parentheses.

same way, undiluted and with just 2-fold dilution, on the Abbott AxSym was provided to us by referring physicians and by Giannopoulos and associates in Australia.<sup>30</sup> In all 17 cases, hCG was clearly determined to be false positive when tested undiluted. The mean result of the 17 cases before dilution was  $100 \pm 35$  mIU/mL (false positive result, mean  $\pm$  SE). In 16 of the 17 cases, hCG was undetectable when serum was diluted just 50% (2-fold) with the Abbott goat serum-containing diluent. In the remaining case it was disproportionately reduced to 8.5 mIU/mL. Clearly, the diluent suppressed the false positive results. This clearly shows that placing the excess animal serum protection in the diluent fails to protect undiluted samples but protect samples as little as 2-fold diluted. This is a very clear defect in the design of the Abbott AxSym total  $\beta$ -hCG test.

We have spoken to the 7 manufacturers of the 8 other principal tests used in the United States and Canada (Table III). From what we are told, all manufacturers add serum or excess nonspecific immunoglobins to constant components of the test pack, to the capture and/or tracer antibody components. This way, protection is present in undiluted and diluted samples. We conclude that the Abbott test is flawed in design, that this flaw has caused more than a 10-fold increase in false positive results than has any other current manufacturer's hCG test and that the test has harmed a large number of women. Whatever happened to the pharmaceutical/diagnostic industry slogan, to always help and never harm people?

It is not only the USA hCG Reference Service that is finding this major false positive problem specifically with the Abbott AxSym test. We found, through a MEDLINE search, that all other recent reports on false positive hCG in the last couple of years involved the Abbott AxSym test and no other hCG test. In 2001, Olsen et al<sup>24</sup> described 2 false positive cases using the Abbott AxSym total  $\beta$ -hCG test. Both patients were assumed to have gestational trophoblastic neoplasia (GTN) and were needlessly treated with chemotherapy. In 2003, Rode et al<sup>26</sup> described 2 false positive cases using the Abbott AxSym total  $\beta$ -hCG test, again involving GTN and needless surgery and chemotherapy. In 2003, Billeux et al<sup>29</sup> identified 2 patients diagnosed and treated with either surgery or chemotherapy for ectopic pregnancy who were needlessly treated because of false positive hCG with the Abbott AxSym total  $\beta$ -hCG test. In 2003, Giannopoulos et al<sup>30</sup> identified 2 women erroneously identified as pregnant because of false positive results with the Abbott AxSym total  $\beta$ -hCG test. In 2003, Trojan et al<sup>31</sup> avoided treatment of a patient for testicular germ cell malignancy by demonstrating false positive hCG results with the Abbott AxSym test. This test urgently needs repair. Until that time, physicians should be very cautious about false positive results when their laboratory is using this test. In the interim, laboratories should limit Abbott AxSym hCG tests to those manually diluted 2-fold to avoid most false positive cases.

### Conclusion

Multiple forms of hCG exist in gestational trophoblastic disease and cancer. Any one of the forms can predominate in serum or urine in gestational trophoblastic disease and cancer, and detection of all these molecules is critical for appropriate management. Two assays appropriately identify all these hCG-related molecules: the DPC Immulite/Immunitite 2000, an automated assay, and the manual UK RIA and similar RIA technologies.

False positive hCG results are a major problem in the management of gestational trophoblastic disease and cancer today. A particular problem is observed with the Abbott AxSym test, which is flawed in design. It should be avoided in the management of gestational trophoblastic disease and cancer. As shown in a blind study, some samples that were false positive with the Abbott AxSym test were also false positive with the UK RIA; none were false positive with the DPC Immulite test. The results clear-

ly show that the DPC Immulite/Immulite 2000 is probably the only appropriate assay for monitoring patients with gestational trophoblastic disease or cancer.

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