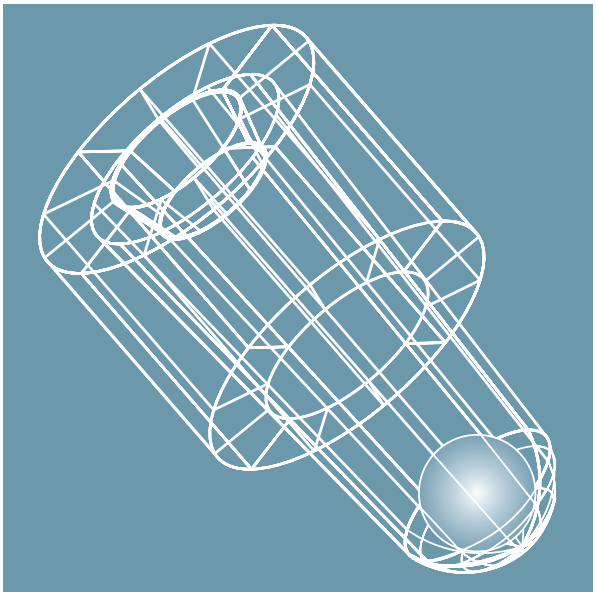
DPC Technical Report

IMMULITE®

HCG and Subunits: DPC Assay Specificities and Clinical Utility in Obstetrical Care and Oncology



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Introduction

Human chorionic gonadotropin (HCG), a protein hormone that stimulates progesterone secretion, appears in biological fluids as intact HCG, free α subunit, free β subunit, and various fragments such as nicked HCG and nicked free β HCG. For more than 25 years, researchers have investigated the molecular forms of HCG in pregnancy and in different physiopathological contexts, including trophoblastic diseases, testicular cancers, and nongonadal and nonplacental tumors. Immunological HCG measurements contribute much to understanding the structure, metabolism and clinical significance of the molecules. Immunoassay specificities for different forms must be identified, however, to produce consistent and reliable results.[1]

Structure of HCG

HCG has two noncovalently associated subunits—α and β—that share about 10 percent sequence identity and fold into similar conformations. The α and β subunits have five and six disulfide bridges, respectively. Synthesis of each subunit appears to be independent. After glycosylation, they are secreted as separate free α and free β subunits or as a dimeric, intact HCG molecule.

Both the pituitary gland and placental tissue express the α subunit (92 amino acids; MW 14.5 kDa), which is immunologically indistinguishable from the α subunits of the other pituitary glycoprotein hormones. The free α subunit appears in the sera of pregnant women, postmenopausal women and patients with a variety of conditions, including hydatidiform mole, choriocarcinoma, carcinoid, pituitary adenoma, pancreatic islet cell tumors, uremia and nonendocrine malignant tumors. The clinical utility of free α subunit measurements remains to be determined.

The β subunit (145 amino acids; MW 22.2 kDa) determines the biological and immunoreactive uniqueness of the intact HCG molecule. While the β subunit of HCG closely resembles the β subunit of LH (the two being identical in 97 of 121 amino acids), β-HCG differs in its serine-rich, extended, carboxyl-terminal (C-terminal) peptide (24 amino acids). Antibodies developed against the C-terminal peptide demonstrate minimal or no crossreactivity with LH. The C-terminal antibodies prove useful in measuring low HCG concentrations present at early stages in pregnancy and in ectopic pregnancies. Patients with malignant tumors have increased free β-HCG levels, suggesting clinical implications for the molecule.[2]

The other identified HCG forms have limited (< 20%) or no biological activity.[3-5] Nicked HCG has a cleavage site in the β chain between amino acids 47 and 48 (or less commonly between positions 43 and 44 or 44 and 45). Circulating nicked HCG levels rise throughout pregnancy, increasing to about 9 percent of the total HCG by the second month and to about 21 percent at term. Circulating nicked free β-HCG appears as well. The main degradation product of β-HCG is the β core fragment, which is measurable only in urine. It goes by a variety of names in the literature, including urinary gonadotropin peptide (UGP). Measurements of the β core fragment may be informative for ovarian, bladder and cervical cancer follow-up therapy. (Representations of the various forms of HCG and its subunits appear in Figure 1.)

Standardization of HCG

The various forms of HCG have posed problems for standardization. In 1975, WHO released three HCG preparations, shown in Table 1. The First International Reference Preparation (1st IRP) 75/735, prepared by Professor Canfield and

Professor Birken from Columbia University, US, contained intact and nicked HCG in a 9-to-1 ratio. In 1986, the 1st IRP changed status to become the Third International Standard (3rd IS) for HCG; it serves as the current standard. The International Federation of Clinical Chemistry recently initiated development of new IRPs for intact, free β subunit, nicked, and other HCG forms.

Table 1. *WHO HCG preparations released in 1975.*

HCG form	WHO Prep. Number	Conversion
Intact	**75/537**	**1 µg = 9.3 IU** **1 IU = 0.0029 nmol HCG**
Free β	**75/551**	**1 µg = 1 IU** **1 IU = 0.045 nmol**
Free α	**75/569**	**1 µg = 1 IU**

Total HCG Assay

Assays that detect intact and nicked HCG forms as well as the free β subunit provide the most useful clinical information. Many immunoassays do not detect nicked HCG forms. Van Ingen (Rotterdam, The Netherlands) demonstrated that IMMULITE® HCG recognizes both the intact and nicked forms (abstract presented at the 1998 annual meeting of the AACC).[6] The researchers spiked pooled serum samples with identical mass concentrations of either HCG or nicked HCG, then measured the HCG concentrations using several immunoassay systems. Table 2 lists the results, expressed as HCG concentration relative to IMMULITE HCG. IMMULITE HCG detects nicked and intact forms, whereas the other automated immunoassays detect only a small percentage of nicked HCG.

Table 2. *Reactivities of several immunoassay systems expressed as HCG concentrations (by mass) relative to IMMULITE HCG (= 100%). (Adapted from van Ingen.)*

Assay	HCG(%)	Nicked HCG(%)
IMMULITE HCG	**100**	**129**
IMx Total β-hCG	**113**	**55.7**
IMx hCG	**79.6**	**11.7**
MAIAclone hCG + β	**91.6**	**4.5**
ACS HCG + B	**74.5**	**12.9**
Elecsys hCG	**87.1**	**3.1**

HCG Levels Throughout Normal Pregnancy

HCG is secreted by the trophoblast in increasing amounts from the time of implantation of the fertilized ovum (blastocyst) in the uterine endometrium. HCG can be detected in serum as early as 6 days after conception, which corresponds to approximately 8 to 10 days after the preovulatory LH surge. HCG concentrations increase rapidly thereafter, reaching peak values at 8 to 9 weeks of gestation. Levels decrease throughout the first part of the second trimester of pregnancy and remain stable until term.

Clinical Utility

HCG has been measured in vaginal fluid as a tool for detecting premature rupture of membranes (PROM) in pregnant women. HCG is secreted by the vaginal glands and is present in low levels in the vaginal fluid of normal pregnant women. If PROM occurs, however, amniotic fluid is released into the vaginal cavity. HCG levels measured in vaginal fluid during the second and third trimester are significantly higher in patients with PROM than in normal pregnant women.[7]

Many publications describe the value of detecting multiple HCG-related molecules in abnormal

pregnancies, aneuploidy (trisomy-21 pregnancies), spontaneous and threatened abortions, preeclampsia, cancers and trophoblastic diseases. (See Table 3.) Reliable monitoring of serum total HCG (including intact HCG, nicked HCG, free β subunit and nicked free β subunit of HCG) is essential in patients who have had moles evacuated. Such monitoring could minimize unnecessary chemotherapy in patients entering remission. It also provides an early indication for additional chemotherapy if HCG becomes or remains detectable.[1,8-13]

Choriocarcinomas, one of the gestational trophoblastic diseases, arise mostly in the uterus from hydatidiform mole, following abortion, or during normal pregnancy. They can also occur in the testis.[14] Although choriocarcinomas represent less than 1 percent of gynecological malignancies, they are important to recognize: early treatment of this life-threatening cancer results in a high cure rate. Metastases are commonly found at diagnosis and are taken into account in disease staging.

Table 3. *Conditions in which HCG measurements have clinical utility.*

Early pregnancy
Ectopic pregnancies (in combination with progesterone)
Miscarriages
Threatened abortions
Preeclampsia
Detection of premature rupture of the membranes (measurement in vaginal fluid)
Trophoblastic diseases (ovary and testis)
Neoplasms
Tumors of the male genital tract
Bladder carcinomas
Risk calculations for trisomy-21 and trisomy-18

HCG and especially the free β-HCG subunit can aid in the diagnosis and monitoring of choriocarcinomas.

In testicular tumors, measurement of HCG in conjunction with α-fetoprotein (AFP) is used as an aid in determining tumor type (seminoma, nonseminoma), prognosis and therapy. These tumor markers are essential monitoring tools; failure of either marker to return to normal after therapy indicates residual tumor.[15] In certain testicular tumors, the ratio of free β-HCG subunit to HCG can be quite high; occasional tumors secrete only the free β subunit and virtually no detectable intact HCG.[16, 17]

Conclusion

HCG circulates in several forms, the proportions of which may vary with patient condition or disease. Commercial assays for HCG have different antibody specificities; these differences must be recognized to achieve consistent and reliable HCG results. The IMMULITE HCG assay measures both the intact and nicked forms of HCG as well as unnicked and nicked free β subunit. The IMMULITE Free Beta HCG assay* is specific for the free β subunit, measuring both the unnicked β and nicked β forms. Cole[1] advises that laboratories be aware of what an HCG assay actually measures. He underscores the importance of measuring multiple forms of HCG and β subunit depending on the clinical context.

* Available outside the US

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IMMULITE Assays

IMMULITE HCG is a solid-phase, two-site, chemiluminescent enzyme immunometric assay designed for the quantitative measurement of HCG in serum. The assay has a calibration range of up to 5,000 mIU/mL, with a detection limit of approximately 1.1 mIU/mL. The intraassay precision (CV) ranges from 3.6% to 5.2% for mean HCG levels between 3,329 and 30.9 mIU/mL, respectively. The interassay precision (CV) ranges from 7.8% to 9.9% for mean HCG levels between 37 and 3,569 mIU/mL, respectively. Reactivity of the β subunit of HCG is approximately 130%. This assay, therefore, measures both intact HCG as well as the free β subunit of HCG. It also measures nicked HCG and nicked free β-HCG. (The IMMULITE® 2000 HCG assay uses the same antibodies as those in the IMMULITE HCG assay and can be expected to demonstrate similar reactivity.) The IMMULITE HCG assay exhibits no high-dose hook effect at HCG levels as high as 2,000,000 mIU/mL. Results from IMMULITE HCG on 54 spiked patient samples correlated well with those obtained by DPC's Coat-A-Count® HCG IRMA.

A study by Vankrieken et al.[18] found that carryover between adjacent samples on the IMMULITE immunoassay analyzer was extremely low (<0.0002%). The IMMULITE HCG assay correlated well with the Serono HCG MaiaClone IRMA on first trimester, ectopic and molar pregnancy samples, and on samples from patients with tumors of the genital tract.

IMMULITE Free Beta HCG* is a solid-phase, two-site, sequential chemiluminescent enzyme immunometric assay designed for the quantitative measurement of the free β subunit of HCG in serum. The assay has a working range of up to 80 ng/mL (IU/L) and a detection limit of approximately 0.02 ng/mL. The intraassay precision ranges from 5.2 to 10% for free β-HCG levels between 0.60 and 63 ng/mL, and the interassay precision is better than 10% for levels as low as 0.6 ng/mL. Crossreactivities of intact HCG, free α-HCG, LH β subunit and TSH β subunit are approximately 0.089, 0.15, 0.4 and 0.002%, respectively. No crossreactivity has been observed with FSH, FSH β subunit or TSH. Due to the binding sites determined by epitope mapping on the free β chain, the IMMULITE Free Beta HCG assay fully recognizes the nicked free β subunit of HCG.

Catalog Numbers:

IMMULITE HCG
- LKCG1 (100 tests)
- LKCG5 (500 tests)

IMMULITE Free Beta HCG*
- LKFB1 (100 tests)
- LKFB5 (500 tests)

IMMULITE 2000 HCG
- L2KCG2 (200 tests)
- L2KCG6 (600 tests)

DPC also offers Coat-A-Count® HCG IRMA which recognizes intact HCG and nicked HCG.

* Available outside the US

Intact HCG and Related Molecular Forms

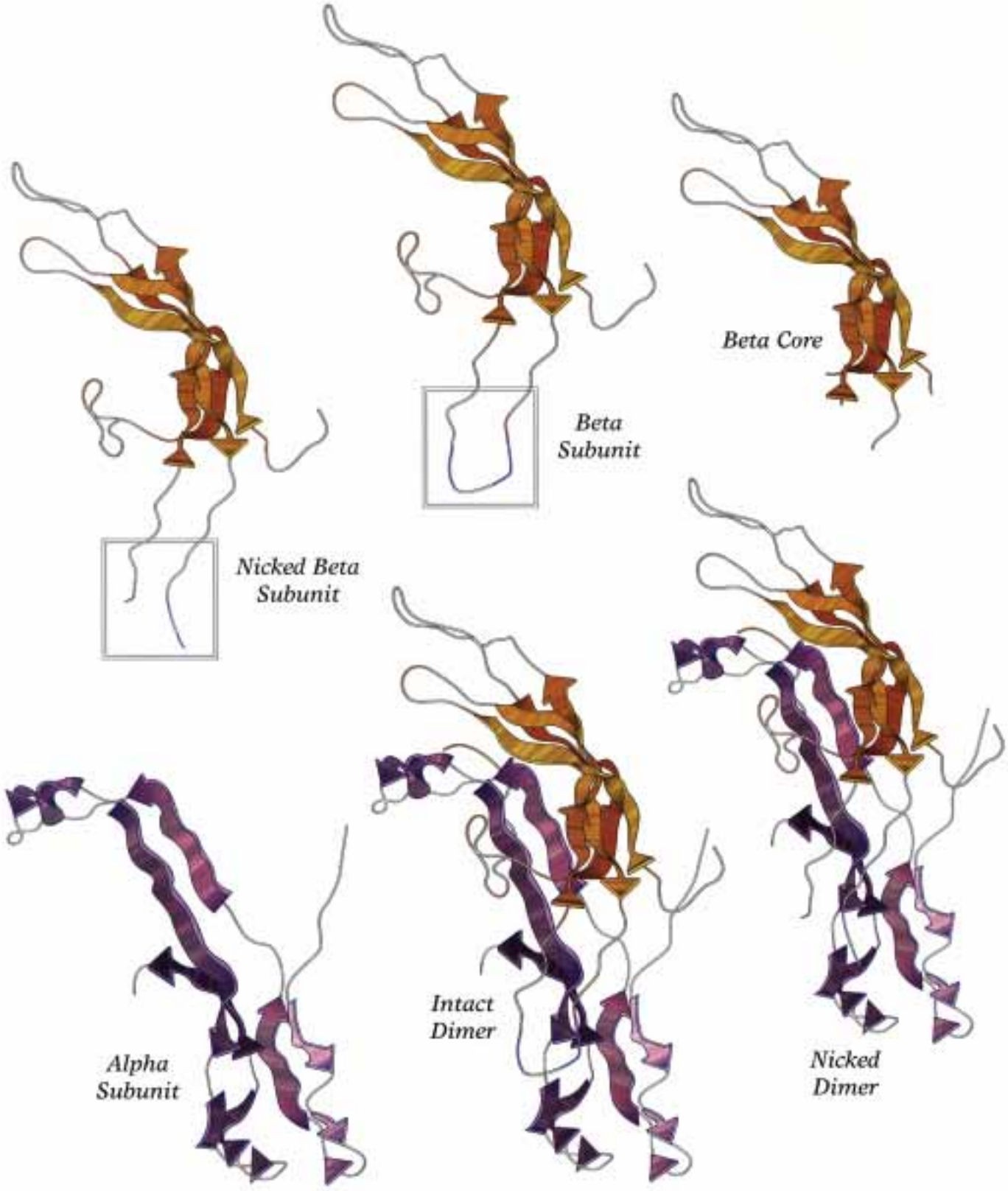

Figure 1. *Among the several HCG forms that appear in body fluids are free α subunit, free β subunit, nicked free β (with cleavage sites—between amino acids 47 and 48, 43 and 44, or 44 and 45—in blue), intact dimer, nicked dimer (includes nicked β subunit), and β core fragment (amino acids 6 – 40 and 55 – 92).*

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