Human chorionic gonadotrophin (serum, plasma; urine)

1 Name and description of analyte

1.1 Name of analyte
Human chorionic gonadotrophin (hCG; referred to by this abbreviation throughout article)

1.2 Alternative names
Chorionic gonadotrophin, choriogonadotrophin. The terms beta-HCG and β-HCG are no longer recommended.

1.3 Description of analyte
hCG is a glycoprotein heterodimer composed of an α- and a β-subunit non-covalently bound to each other. It is contains 237 amino acids with a molecular weight of approximately 38 kDa and a carbohydrate content of 20%; the α- subunit has 92 amino acids and the β- subunit, 145 amino acids. The α-subunit is common to luteinising hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), while the β-subunit is unique to hCG. hCG is synthesised by trophoblastic tissue of the placenta in pregnancy, although it can be produced by other cells in various disease states (see 7.1). Synthesis of the two subunits is under individual genetic control. In early pregnancy, the free β-subunit is produced together with an intact molecule of hCG; later in pregnancy, an excess of free α-subunit is produced. In certain cancers, differential production of subunits is observed. Nicked forms of hCG are generated by metabolism (see 4.5); the β core fragment (β-subunit residues 6-40 disulfide-linked to residues 55-92) is the main form found in urine. hCG has a plasma $t_{1/2}$ of 24–36 h.

1.4 NLMC code
To follow

1.5 Function of analyte
The principle action of hCG is to promote progesterone production by the corpus luteum in the ovaries during the first trimester of pregnancy (up to weeks 10–12); in turn, this progesterone maintains the endometrium. After the first trimester, the placenta takes over from the corpus luteum in synthesising progesterone. hCG also has other functions, including promoting angiogenesis in the uterine wall and an immunosuppressive effect to block the phagocytosis of invading trophoblast cells. hCG also provides stimulation for male sexual differentiation by stimulating testosterone synthesis in the testes of male fetuses. The presence of hCG in the plasma at other times can indicate abnormal trophoblastic tissue or a tumour secreting the hormone ectopically.

2 Sample requirements and precautions

2.1 Medium in which measured:
- hCG can be measured in plasma and serum
- hCG can also be detected in urine as a point-of-care test (PoCT) for pregnancy: early morning urine samples are preferred for this purpose.

Other media in which hCG can be measured but which are not considered further include the following.

- Amniotic fluid: highest concentrations are found between weeks 8–10 of gestation at 68,100 IU/L ± 8,422 ($\bar{x}$ ± SD). The concentration decreases to 2005 ± 260 IU/L from week 18. Concentrations correlate with those in maternal plasma owing to direct diffusion from the placenta.
- Peritoneal fluid: in ectopic pregnancy, hCG concentration in peritoneal fluid exceeds that in plasma with a ratio >1.1 in tubal pregnancy compared to normal pregnancies where the ratio is 0.24–0.87.
- Cerebrospinal fluid (CSF): hCG is a large peptide hormone which does not cross the blood-brain barrier. High concentrations in the CSF can indicate the presence of tumour metastases to the brain. Serial measurements may indicate the response to therapy response in such patients, although imaging is now preferred for such assessment.

2.2 Precautions re sampling, handling etc.

Plasma/serum hCG can be measured in serum separator tubes, heparin tubes or EDTA tubes. Human chorionic gonadotrophin is stable for 48 h at room temperature, 3 days at 4–8 °C and 12 months at -20 °C. Adequate centrifugation to remove fibrin and particulate matter etc. is required, as these provide a source of error. Heat-inactivated samples should not be used and multiple freeze-thaw cycles of specimens should be avoided. Specimens must be at room temperature before analysis.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Serum/plasma hCG measurements are used:

- to diagnose early pregnancy (though this is usually done by PoC urine testing)
- to monitor early pregnancy (ectopic, threatened abortion). NB that following natural termination of pregnancy, [hCG] should fall
- as a component in combined and triple tests for Down's syndrome and for screening for chromosomal abnormalities (trisomy 21)
- to monitor patients with gestational trophoblast tumours (hydatidiform moles and choriocarcinoma)
- to diagnose and monitor patients with germ cell tumours (testicular cancer in men and extragonadal germ cell tumours).

Urine hCG is used for routine pregnancy testing, typically at the time of the first missed menstrual period.

Note that hCG (given i.v.) can be used to test Leydig function in men with primary hypogonadism.

3.2 Limitations

- A very low serum [hCG] does not exclude pregnancy.
- False negative results can occur in very early or abnormal pregnancy.
- A false negative result from urinary analysis may occur in early pregnancy due to diuresis (e.g. requirement for a full bladder prior to ultrasound).
- An ectopic pregnancy cannot be diagnosed using [hCG] alone.

- hCG can be produced by non-germ cell tumours (e.g. small cell carcinoma of lung).
- In post-menopausal women on dialysis, [hCG] can be up to ten times the upper reference limit due to reduced renal excretion of physiologically produced hCG.
- The pituitary gland is source of hCG in post-menopausal women with a normal range of 9.7 ± 6.5 IU/L ($\bar{x}$ ± SD).
- Normal testes are capable of producing small amounts of hCG (serum concentrations up to 5 IU/L).
- If a result greater than the corresponding reference range is obtained (see 5.1.1), the test should be repeated using an alternative method, or a second sample taken after 48 h should be tested. [hCG] must be interpreted in light of clinical knowledge and pathological condition.
- Because of the existence of multiple forms of hCG and their varying detection by different assays, it is important to be aware of the stated characteristics of the assay used. Assays for oncological use should ideally detect all forms of hCG.

4 Analytical considerations

4.1 Analytical methods

1. Serum

hCG in serum can be measured using a sandwich immunoassay format with two monoclonal antibodies against different epitopes; these are a solid phase capture antibody and a labelled antibody for detection. Different assays measure intact hCG and/or the β-subunit. Separate antibodies for the α- and β-subunit are used to measure only the intact molecule. Antibodies raised against determinants of the β-subunit alone measure both the intact hCG molecule and free β-chains. For oncological purposes, hCG assays should ideally measure all forms of hCG (including nicked forms)as some tumours (e.g. seminomas, choriocarcinomas) may only secrete free β-chains or nicked forms. Down's screening requires measurement of β-subunit only. Radioimmunoassays are rarely used now owing to the health and safety risks associated with using radioactively labelled substances.

- The Abbott Architect® assay is a two-step chemiluminescent microparticle immunoassay. It is approved for detection of early pregnancy only.
- The ADVIA Centaur® assay is a chemiluminometric two-site sandwich immunoassay. Two antibodies are used; goat anti-hCG antibody labelled with acridinium ester and mouse anti-hCG antibody that is covalently coupled to paramagnetic particles.
- The Roche electrochemiluminescence immunoassay 'ECLIA'® combines a sandwich antigen-antibody reaction with two hCG-specific monoclonal mouse antibodies (biotinylated and ruthenium-labelled) on the surface of a magnetic microbead.
- The Micro-ELISA® total assay uses a solid phase rabbit polyclonal antibody and mouse monoclonal antibody-enzyme horseradish peroxidase conjugate, both raised against β-HCG.
- The AutoDELFIA® hCG assay uses time-resolved fluoroimmunoassay. The assay has been designed to be suitable for screening of Down's syndrome during the second trimester of pregnancy. A solid phase mouse anti-β hCG

antibody and a europium labelled mouse anti-αhCG antibody are used. Enhancement solution dissociates europium ions from labelled antibody forming highly fluorescent chelates which are then measured.

- The Brahm's Free βhCG KRYPTOR immunoassay is designed to use hCG as an in vitro tumour marker and for risk-assessment of foetal chromosomal abnormalities. The method uses Time-Resolved Amplified Cryptate Emission (TRACE) technology which is based on a non-radioactive energy transfer from a donor antibody to an acceptor antibody as a result of a completed immune reaction. The signal emitted is temporally delayed fluorescence.

2. Urine

PoCT strips are qualitative, with a detection limit <25 IU/L, and use the sandwich ELISA principle. The sample is drawn up the capillary test strip to the reaction zone. In a positive urine sample, hCG binds monoclonal anti-hCG Ab-enzyme conjugates. These complexes are carried along the strip to the test zone where hCG binds to solid phase capture polyclonal hCG antibodies. The fixed enzymes then activate the dye substrate in the test zone. In a negative urine sample, soluble anti-hCG Ab-enzyme conjugates are dissolved and carried along strip by capillary flow. At the test zone, the antibody-enzyme conjugates lacking bound hCG flow past the immobilised polyclonal hCG antibodies and dye substrate and the dye substrate is not activated.
In both negative and positive urine samples, the sample flows to the control zone where the monoclonal mouse conjugates binds the immobilised (goat) anti-mouse antibodies and the dye substrate is activated. Urine devices should be able to detect hyperglycosylated hCG, which is the main form in early pregnancy.

4.2 Reference method

No reference method is available at present for serum/plasma analysis. An evaluation into the feasibility of developing a LC-tandem MS technique is currently being undertaken. PoCT devices are evaluated against hCG immunoassay.

4.3 Reference materials:

1st reference reagents for hCG, 2001 NIBSC: National Institute for Biological Standards and Control, Washington DC, USA.		
Preparation (and material)	**Held at**	**Code**
Intact hCG (purified to remove nicked forms and free subunits)	NIBSC	99/688
α-hCG subunit (purified α-subunit)	NIBSC	99/720
β-core fragment hCG (residues β6-40 disulphide bonded to β55-92)	NIBSC	99/708
β-hCG subunit	NIBSC	99/650
Nicked hCG subunit (purified to remove intact dimeric hCG, α-subunit and nicked β-subunit)	NIBSC	99/642
Nicked β-hCG subunit (partially degraded β-subunit, missing peptide bonds in the β40-50 region)	NIBSC	99/692

4.4 Interfering substances

For the electrochemiluminescent method, no significant interference is reported from bilirubin up to 410 μmol/L, haemolysis up to 1 g/dL, lipaemia up to 15 mmol/L, and biotin <327 nmol/L. No significant cross-reactivity is detected with an LH <1000 IU/L. [hCG] may be falsely elevated or lowered due to (but not limited to) non-specific protein binding and medications such as exogenous hCG (Profasi, Pregnyl, Novarel); these can affect the entire range of the assay.

4.5 Sources of error

1. Trophoblastic tumours secrete nicked hCG in addition to normal hCG, which is not recognised by all hCG assays. These forms can have variations in peptide structure and linkages between the α- and β-subunits, and are differentially glycosylated and sialylated.
2. A high-dose hook effect at concentrations >750,000 IU/L may result in falsely low values and dilution of sample is required. A high dose hook effect can also occur using PoCT devices. hCG concentrations can differ depending on the laboratory method used: values measured using different techniques cannot be directly compared. If [hCG] values are being used for monitoring purposes using different methods, parallel measurements using both methods should be made.
3. Erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes. This can also occur in patients who are routinely exposed to animals or animal serum products, and who develop heterophilic antibodies.

5 Reference intervals and variance

5.1.1 Reference interval (adults)

Reference intervals for plasma [hCG] in different patient populations using Centaur® assay. N.B. gestational week ranges may vary depending on method used.

Population	**Approximate hCG (IU/L)**
Men	<5
Women	
- non-pregnant: premenopausal	<5
postmenopausal	<10
Pregnancy	*Expected*
Diagnostic concentration	≥10
<1 week	5–50
1-2 week	50–500
2-3 week	100–5,000
3-4 week	500–10,000
4-5 week	1,000–50,000
5-6 week	10,000–1000,00
6-8 week	15,000–200,000
2-3 months	10,000–100,000

5.1.2 Reference intervals (others): β-hCG <0.2 IU/L

5.1.3 Extent of variation

5.1.3.1 Inter-individual CV: not applicable. [hCG] varies greatly between individuals and reference intervals during different gestational stages cannot be assigned precisely. Determination of the rate of increase over a defined time interval is more appropriate to monitor for normal pregnancy than a single measurement (see 7.3.1(1)).

5.1.3.2 Intra-individual CV: insufficient data

5.1.3.3 Index of individuality: insufficient data

5.1.3.4 CV of serum method: 4% at 3 IU/L
3% at 390 IU/L
CV of Abbott urine method: 4% at 31 IU/L
3.9% at 181 IU/L

5.1.3.5 Critical difference: insufficient data [See 5.1.3.2]

5.1.3.6 Sources of variation

Increased [hCG] can also occur in non-pregnant peri-menopausal or post-menopausal women, in patients with gestational trophoblastic disease or other tumours, following previous injection of hCG, and in patients with heterophilic antibodies.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation

1. Early diagnosis of pregnancy

hCG is detectable in maternal blood 7–9 days after conception and in urine 1–2 days after that, with its concentration doubling thereafter every 48 h. Maximum [hCG] is reached at approximately week 10 of gestation and subsequently declines (although it remains detectable in the urine throughout pregnancy (see 5.1.1)). Qualitative testing of urine for hCG is used routinely for diagnosis of pregnancy and is reliable from at least 10 days after a missed menstrual period. It should only be followed up using laboratory serum/plasma testing if there is believed to be is an issue with the pregnancy, for example if there is vaginal bleeding, abdominal pain or if a gestational sac is not visible on ultrasound. Pregnancy is indicated by a serum [hCG] of >10 IU/L. In multiple pregnancies, [hCG] is higher, doubling time is shorter, and the absolute concentration is higher after week 10 of gestation.

2. Monitoring in early pregnancy (for suspected ectopic pregnancy or spontaneous abortion)

Ectopic pregnancy should be suspected when serum [hCG] is >1000 IU/L but an intrauterine sac cannot be visualised on a trans-vaginal ultrasound. The rate of [hCG] increase is important; in early normal pregnancy, [hCG] doubles every 2.5–3 days whereas there is usually a slower rate of increase in ectopic pregnancy.

3. Screening for Down's syndrome

The NHS screening programme for Down's syndrome includes measurement of serum hCG and incorporation with other parameters in the triple or combined test. A risk score of the fetus having Down's syndrome is then calculated. Maternal [hCG] is an average of two times higher when fetal Down's syndrome is present. Biochemical screening is not diagnostic but allows selection of a high-risk group for which further investigation is indicated.

4. Follow-up and monitoring of patients with gestational trophoblast tumours
These conditions are caused by abnormal growth of cells within the uterus ranging from hydatidiform moles (molar pregnancy) to choriocarcinoma. hCG concentrations of up to 2 x 10^6 IU/L have been observed in trophoblastic tumours, and an initial value >400,000 IU/L is a high risk factor for treatment failure. hCG measurement is vital for successful management because concentrations correlate with tumour volume.
5. Diagnosis, follow-up and monitoring of germ cell tumours (seminomas, non-seminomatous germ cell tumours (NSGCT), combined tumours, extragonadal tumours)
hCG should be used in combination with α-fetoprotein to detect and stage germ cell tumours. These proteins are produced independently and relate to different tumour types. It is essential to distinguish seminomas from teratomas as they are treated differently.

- In seminomas, [hCG] is elevated in 10–30% of patients who have syncytiotrophoblastic cells in the tumour, with serum concentrations ranging from 10–2000 IU/L.
- [hCG] in CSF is markedly elevated in primary intracranial germ cell tumours compared to serum, as hCG does not cross the blood-brain barrier.
- In NSGCT, [hCG] is elevated and concentrations range from 5–1000 IU/L. Prevalence of elevated [hCG] depends on tumour stage (stage I 45%, II 55%, III 84%).
- [hCG] is not elevated in differentiated teratomas or yolk sac tumours.

(6. hCG is given by injection to test Leydig cell function in men with primary hypogonadism.)

6.2. Confounding factors
See 3.2 & 5.1.3.6

7 Causes and investigation of abnormal results

7.1 High values
7.1.1 Causes
1. Pregnancy
2. Down's syndrome pregnancy
3. Placenta trophoblastic tumours (hydatidiform moles, choriocarcinomas)
4. Germ cell tumours (seminoma, NSGCT)
5. Extragonadal germ cell tumours

The table indicates various malignant diseases in which [hCG] is elevated.

Prevalence (%) of elevated hCG and β-hCG	
Tumour	**(%)**
Testicular/placental choriocarcinoma	100
Hydatitiform mole	97
NSGCT	48–86
Seminoma	10–22
Pancreatic adenocarcinoma	11–80
Islet-cell carcinoma	22–50

Gastric cancer	0–52
Ovarian cancer; epithelial	18–41

7.1.2 Investigation

- The gestational age of a healthy pregnancy can be determined in the first seven weeks using at least two hCG measurements from separate samples collected 2–7 days apart. Doubling time is 2.5–3 days at this stage and log[hCG] is plotted against measurement time.
- In Down's screening, patients identified as high risk are offered amniocentesis or chorionic villous sampling for diagnosis of having a Down's baby.
- Following surgical evacuation of the uterus, trophoblastic tumours are monitored by serial hCG measurements (weekly until three weeks after normalisation, monthly for six months and yearly thereafter). If chemotherapy is required (i.e. for choriocarcinoma), response is monitored by serial measurements. Treatment is continued until six weeks after [hCG] normalisation. Yearly measurement is recommended to detect relapse. [hCG] should be within the reference range for ≥6 months for patients wanting to conceive. After any further pregnancy, [hCG] should be measured to exclude disease recurrence.
- Patients with clinical findings consistent with a germ cell tumour (testicular lump, abdominal mass) and elevated [hCG]and/or [α-fetoprotein] (AFP) require immediate referral to a specialist centre. A prognosis staging system has been proposed by the International Germ Cell Cancer Collaborative Group (see 9.1). Before surgery, it is essential to measure [hCG] to determine rate of fall post-surgery/ chemotherapy. A transient increase of [hCG] immediately following chemotherapy is due to lysis from tumour tissue. It is estimated up to 100,000 tumour cells may still be present at 1 IU/L [hCG]. As a result, chemotherapy should be continued until [hCG] becomes undetectable. Frequent monitoring (weekly) is recommended until [hCG] is normalised (see 9.1 SIGN guidelines) and long term monitoring following treatment should be in accordance with clear clinical protocols.

7.2 Low values

7.2.1 Causes

- Threatened or missed abortion
- Ectopic pregnancy
- Inter-uterine death

7.2.2 Investigation

In the conditions mentioned above, serial serum hCG values either show a slow rise or decline prematurely. Determining the cause of low values require additional investigation e.g. transvaginal ultrasound, laparoscopy, in addition to high levels of clinical suspicion, as based on the presence of, for example, abdominal pain or vaginal bleeding. To aid in diagnosis of ectopic pregnancy, at least two [hCG] measurements should be obtained from samples collected 1–7 days apart. If the log-linear slope of log[hCG])/time (in log(IU/L)/day) >0.11, normal pregnancy is likely compared to ectopic pregnancy/spontaneous abortion where the slope is <0.11. Serum [progesterone] has a lower absolute concentration in ectopic pregnancy, and is

used in combination with [hCG]. Serum progesterone >25 nmol/L is suggestive of viable intrauterine pregnancy.

7.3 Notes

Diagnosis of gestational trophoblastic disease is made by ultrasound or histological examination of tissue after an episode of vaginal bleeding during early pregnancy. [hCG] measurement is required for monitoring and follow-up of therapy only. When monitoring germ cell tumours, [hCG] can be transiently increased by the use of cannabis. Screening for germ cell tumours using [hCG] is not recommended.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

- Urine PoCT ranges from 77–100% specificity, 31–100% sensitivity according to manufacturer's kit inserts.
- Ectopic pregnancy: log[hCG]/time(days) >0.14; sensitivity 99%, specificity 65% to distinguish between normal inter-uterine and ectopic pregnancy. A normal rise has a positive predictive value of 94.7% for normal pregnancy.
- Down's screening: at a risk cut-off of 1 in 250 for triple test (hCG, alpha-fetoprotein, unconjugated estriol) = detection rate 67%, sensitivity 61%, false positive rate 5%. At a risk cut-off of 1 in 150 for combined test (hCG, placenta associated plasma protein A (PAPP-A) and nuchal translucency), detection rate 84%, false positive rate 2.2%.
- Choriocarcinoma: sensitivity 99%, specificity 99% in patients suffering from a previous molar pregnancy. Measurement in specialist referral centres with hCG methods to detect hCG and its major isoforms is necessary.
- Metastatic germ cell tumour – Losa Gaspa *et al*. 2002 found hCG had intermediate sensitivity and specificity for detection (67% and 75%, respectively). Losa Gaspa F, Germa JR, Albareda JM *et al*. Metastatic cancer presentation. Validation of a diagnostic algorithm with 221 consecutive patients, Revista Clinica Espanola. 2002;202: 313-319.

9 Systematic reviews and guidelines

9.1 Systematic reviews

1. Menon S, Colins J, Barnhart KT. Establishing a human chorionic gonadotropin cut-off to guide methotrexate treatment of ectopic pregnancy: a systematic review. *Fertil Steril.* 2007;87:481-484. This r*eview concludes that in patients suffering from ectopic pregnancy with [hCG] > 5000 IU/L, methotrexate should be used with caution).*
2. Cao ZT, Rej R. Are laboratories reporting serum quantitative hCG results correctly? *Clin Chem* 2008;54:761–764. *This review discusses how hCG nomenclature can cause confusion in addition to their being a lack of awareness of the different forms of hCG and what forms different assays measure.*

9.2 Guidelines

1. NICE and Antenatal care: routine care for the healthy pregnant woman. http://www.nice.org.uk/nicemedia/pdf/CG6_ANC_NICEguideline.pdf *This summary refers to hCG in reference to antenatal screening. It does not look at women with certain medical conditions or women who develop a health problem during their pregnancy.*
2. Fetal Anomaly Screening Programme - Screening for Down's Syndrome: UK National Screening Committee Policy recommendations 2007 – 2010: Model of Best Practice. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_084731.pdf
3. The management of gestational trophoblastic disease – Royal College of Obstetricians and Gynaecologists. http://www.rcog.org.uk/files/rcog-corp/GT38ManagementGestational0210.pdf
   Guidelines refer to hCG measurement for diagnosis and follow-up of gestational trophoblastic disease.
4. Management of adult testicular germ cell tumours – Scottish Intercollegiate Guidelines Network. http://www.sign.ac.uk/pdf/sign124.pdf
   Guidelines state the use of hCG as a good detector of residual NSGCT after orchidectomy, although it is not specific for NSGCT and can be increased in several other non-germ cell tumours. Guidelines state timeframe for serial hCG measurements prior to and after surgery).
5. NICE and Metastatic malignant disease of unknown primary origin. http://www.nice.org.uk/nicemedia/live/13044/49867/49867.pdf
   This summary refers to hCG as a tumour marker in presentations compatible with germ-cell tumours, especially in mediastinal, retroperitoneal masses and young men. Measurement should also be used in the presence of midline nodal disease.

9.3 Recommendations

1. International Germ Cell Consensus Classification: a prognostic factor-based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group. *J Clin Oncol* 1997;15:594-603. *IGCCCG classify patients with metastatic NSGCT into three prognostic groups using nadir tumour marker concentrations (hCG, AFP,LDH) post surgery, primary tumour site and metastatic sites.*

10 Links

10.1 Related analytes

None

10.2 Related tests

1. α- Fetoprotein: an oncofetal protein used in prenatal diagnostics, and used as a tumour marker for diagnosis and monitoring of certain malignant tumours. Reference interval <44 μg/L.
2. Progesterone: a steroid hormone that exerts several effects on the uterus endometrium for preparation of implantation by the fertilised ovum. Reference interval for female mid-luteal phase (day 21–23) >35 nmol/L.

3. Testosterone: an anabolic steroid hormone required for development of male secondary sexual characteristics and for spermatogenesis. Reference interval (females) 0.1–1.8 nmol/L, (males) 7.6–31.0 nmol/L
4. Down's screening: the combined test offered in the first trimester of pregnancy measures hCG and PAPP-A in serum, and nuchal translucency obtained from ultrasound scan. In the second trimester, the triple test is offered, which measures AFP, hCG and unconjugated estriol concentration in serum.

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