

CHORIONIC GONADOTROPIN (hCG) ELISA

EU:   CAN:  USA: For Research Use Only. Not for Use in Diagnostic Procedures

REF: CAN-HCG-4120 Version: 9.0
Effective: September 13, 2018

INTENDED USE

For the direct quantitative determination of Chorionic Gonadotropin by enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for hCG is immobilized onto the microplate and another monoclonal antibody specific for a different region of hCG is conjugated to horse radish peroxidase (HRP). hCG from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of hCG in the sample.

A set of standards is used to plot a standard curve from which the amount of hCG in patient samples and controls can be directly read.

Please note that a two-step procedure is included and is to be used for assaying serum from pregnant women.

CLINICAL APPLICATIONS

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the placenta. During normal pregnancy the level of this hormone in serum and urine gradually increases up to about the eighth week of pregnancy. hCG has two subunits, namely α and β . The α subunit is similar to the α subunit of the anterior pituitary gland glycoprotein hormones, namely, LH, TSH and FSH. However, the β subunits of these hormones are different. Therefore, this uniqueness distinguishes one hormone from the other, hence specificity. In raising this monoclonal antibody, the immunogen used was β hCG, which helps to make the DBC assay system very specific and sensitive.

In normal pregnancy the increase in hCG starts at about the 5th day after conception and continues to rise until it reaches a maximum at about the eighth week. In some pathological conditions the level of hCG in serum and/or urine is increased. It is a well known fact that hCG is also a tumour marker which is very important in the diagnosis of choriocarcinoma. In the case of hydatiform mole, hCG is also elevated. In about 50% of patients with testicular teratomas the level of hCG is elevated. It is also relevant to note that hCG is a good indicator, in order to follow the response to treatment.

PROCEDURAL CAUTIONS AND WARNINGS

- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

- Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- A calibrator curve must be established for every run.
- The controls should be included in every run and fall within established confidence limits.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

- All the reagents within the kit are calibrated for the direct determination of hCG in human serum. The kit is not calibrated for the determination of hCG in saliva, plasma or other specimens of human or animal origin.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
- Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency

Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes to dispense 25, 50, 100 and 300 μ L
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

- Mouse Anti-hCG Antibody-Coated Break-Apart Well Microplate — Ready To Use**
Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2–8°C.
Stability: 12 months or as indicated on label.
- Mouse Anti-hCG Antibody-Horseradish Peroxidase (HRP) — Requires Preparation $\times 50$**
Contents: Anti-hCG monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 300 μ L/vial
Storage: Refrigerate at 2–8°C.
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:50 in assay buffer before use (eg. 40 μ L of concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 μ L of concentrate in 12 mL of assay buffer. Discard any that is left over.
- hCG Calibrators — Ready To Use**
Contents: Six vials containing hCG in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of hCG. Calibrated against World Health Organization (WHO) 3rd IS 75/589.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 IU/L	2.0 mL
Calibrator B	2.5 IU/L	0.5 mL
Calibrator C	10 IU/L	0.5 mL
Calibrator D	25 IU/L	0.5 mL
Calibrator E	100 IU/L	0.5 mL
Calibrator F	500 IU/L	0.5 mL

Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready To Use

Contents: Two vials containing hCG in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of hCG. Refer to vial labels for the acceptable range.

Volume: 0.5 mL/vial

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation $\times 10$

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. Assay Buffer — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 25 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

7. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

8. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 6 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

ONE-STEP ASSAY PROCEDURE
Specimen Pretreatment: None .
All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.
<ol style="list-style-type: none"> 1. Prepare working solution of the anti-hCG-HRP conjugate and wash buffer. 2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator. 3. Pipette 25 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate. 4. Pipette 100 µL of the anti-hCG-HRP conjugate into each well. (We recommend using a multichannel pipette.) 5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. 6. Wash the wells <u>3 times</u> with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.) 7. Pipette 100 µL of TMB substrate into each well at timed intervals. 8. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD). 9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7. 10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution. <p>* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.</p>

CALCULATIONS

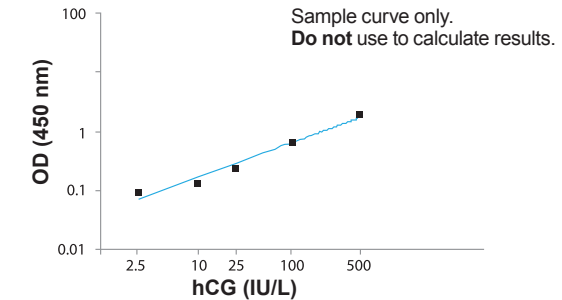
1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the “0” calibrator from the mean absorbance values of the calibrators, controls and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 500 IU/L then dilute it with calibrator A at a dilution of no more than 1:20. The result obtained should be multiplied by the dilution factor.

TWO-STEP ASSAY PROCEDURE
Use this procedure when assaying serum from pregnant women to avoid a high dose hook effect. Specimen Pretreatment: None .
All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.
<ol style="list-style-type: none"> 1. Follow steps 1–3 of the one-step assay procedure. 2. Pipette 100 µL of assay buffer into each well. (We recommend using a multichannel pipette.) 3. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. 4. Wash the wells <u>3 times</u> with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.) 5. Pipette 100 µL of the anti-hCG-HRP conjugate into each well. (We recommend using a multichannel pipette.) 6. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. 7. Wash the wells again in the same manner as step 4. 8. Pipette 100 µL of TMB substrate into each well at timed intervals. 9. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD). 10. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 8. 11. Read the plate on a microplate reader at 415 nm within 20 minutes after addition of the stopping solution.

TYPICAL TABULATED DATA
Sample data only. Do not use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (IU/L)
A	0.075	0.073	0.074	0
B	0.093	0.091	0.092	2.5
C	0.143	0.146	0.145	10
D	0.250	0.267	0.259	25
E	0.711	0.727	0.719	100
F	2.452	2.545	2.499	500
Unknown	0.133	0.130	0.132	7.7

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS SENSITIVITY
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DBC Direct hCG ELISA kit is **0.7 IU/L**.

SPECIFICITY (CROSS-REACTIVITY)
The specificity of the Direct hCG ELISA kit was determined by measuring the apparent hCG value of calibrator A spiked with the following compounds:

Substance	Concentration Range	Apparent hCG Value (IU/L)
hFSH Calibrated against WHO 1st IS 83/575	100–4000 IU/L	< 5.0
hLH Calibrated against WHO 2nd IS 80/552	50–200 IU/L	< 5.0
hTSH Calibrated against WHO 2nd IS 80/558	50–750 mIU/L	< 5.0

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same calibrator curve. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	4.52	0.24	5.3
2	18.80	0.60	3.2
3	121.84	5.36	4.4

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV %
1	5.24	0.36	6.9
2	14.68	0.72	4.9
3	140.27	11.50	8.2

RECOVERY
Spiked samples were prepared by adding defined amounts of hCG to three patient serum samples (1:1). The results (in IU/L) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	3.2	-	-
+ 15	8.4	9.1	92.3
+ 50	23.4	26.6	88.0
+ 250	145.6	126.6	115.0
2 Unspiked	3.3	-	-
+ 15	8.7	9.2	94.6
+ 50	23.9	25.0	95.6
+ 250	142.0	126.7	112.1
3 Unspiked	3.9	-	-
+ 15	9.2	9.5	96.8
+ 50	24.5	27.0	90.7
+ 250	148.0	127.0	116.5

LINEARITY
Two patient serum samples were diluted with calibrator A.

The results (in IU/L) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	67.7	-	-
1:5	12.1	13.5	89.6
1:10	6.2	6.8	91.2
1:20	3.5	3.4	102.9
2	137.7	-	-
1:5	30.2	27.5	109.8
1:10	14.6	13.8	105.8
1:20	7.6	6.9	110.1

REFERENCE VALUES
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (IU/L)
Males	< 5
Females: Non-Pregnant	< 5
Pregnant	> 250

REFERENCES

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SYMBOLS

European Conformity	In vitro diagnostic device	Consult instructions for use
Contains sufficient for <n> tests	Storage Temperature	Legal Manufacturer
Use by	Catalogue Number	Authorized representative
Lot number	Dilute 1: # Before use	