ßhCG [^{125}I] IRMA KIT

(REF: RK-760CT)

The ^{125}I-ßhCG IRMA system provides direct quantitative *in vitro* determination of human Chorionic Gonadrotrophin (hCG) in human serum. ßhCG can be assayed in the range of 0-1000 mIU/ml using 50 µl serum samples.

Introduction

Human Chorionic Gonadrotrophin (hCG) is a glycoprotein with a molecular weight of 38000, secreted by the placenta. Like other glycoprotein hormones (hLH, hTSH and hFSH), hCG contains two different subunits, an α- and a β-chain, linked by noncovalent bindings. The primary structures of the α subunits of these hormones are virtually identical, while their β subunits, responsible for the immunological and biological specificity, are different. Thus a specific determination of hCG can only be made by the determination of its β component. The measured hCG content results almost exclusively from intact hCG molecules but there can be a contribution from the free βhCG subunit.

hCG appears in the sera of pregnant women five days after the implantation of blastocyst and its concentration continually increases until the third month of the pregnancy. The maximum concentration can reach values up to 100 IU/ml. Then the hormone level drops to 25 IU/ml and stays around this value until the last trimester.

Elevated hCG concentrations can be seen in the case of trophoblastic and nontrophoblastic neoplasia, and choriocarcinoma.

Ectopic hormone production which is frequently associated with the metastatic breast cancer and with tumours of the liver, stomach, lung, and uterus often results in the elevated hCG concentration both in men and in non pregnant women.

The current sandwich IRMA system is particularly matched to the direct determination of neoplastic βhCG, whilst gestational βhCG levels can be measured after the pre-dilution of patient sera.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the ßhCG molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 2-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of ßhCG, the unknown concentration of ßhCG in patient samples can determined.

Contents of the kit

1. 1 bottle TRACER (21 ml), ready to use, containing about 980 kBq ^{125}I-anti-ßhCG and capture anti-ßhCG antibody in buffer with red dye and 0.1 % NaN_3.

2. 6 vials STANDARD (6 x 0.5 ml), containing (S1-S6) 0, 5, 20, 80, 300, 1000 mIU/ml ßhCG (3d IRP Int. Std.) in serum with 0.1% NaN_3.

3. 1 vial CONTROL SERUM. 1.0 ml human serum with 0.1% NaN_3. The concentration of the control serum is specified in the quality certificate enclosed.

4. 1 bottle DILUTION SERUM. 15 ml serum with 0.1 % NaN_3.

5. 2 boxes COATED TUBE, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

6. 1 bottle WASH BUFFER CONCENTRATE (20 ml), containing 0.1% NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter

Recommended tools and equipment

repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-ml pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens.

For normal pregnancy, even during the first several weeks of gestation, the serum intact hCG levels may increase above 1000 mIU/ml. To avoid reassaying samples containing high levels of hCG , samples should be diluted 1:250 with the dilution serum enclosed.

The recommended dilution procedure is the following:

Step A: 10 µl sample + 90 µl dilution serum (1:10 dilution)

Step B: 10 µl of the 1:10 diluted sample from step A + 240 µl dilution serum (final 1:250 dilution).

Preparation of reagents, storage

Add the wash buffer concentrate (20 ml) to 700 ml distilled water to obtain 720 ml wash solution. Upon dilution store at 2-8°C until expiry date.

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide , refer to Table 1.)

1. Equilibrate reagents and samples to room temperature before use.
2. Label coated tubes in duplicate for each standard (S1-S6), control serum and samples.
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 50 µl of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
5. Pipette 200 µl of tracer into each tube.
6. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
7. Incubate tubes for 2 hours, shaking at room temperature.
8. Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
9. Return the tube-rack to an upright position, and repeat step-8 two more times.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the ßhCG concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**50**		
Control			**50**	
Sample				**50**
Tracer	**200**	**200**	**200**	**200**
Shake for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{2\text{-}6} / C / M_x \text{ (cpm)} - S_1\text{(cpm)}}{T\text{(cpm)}} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of ßhCG.

Determine the ßhCG concentration of the unknown samples by interpolation from the

standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	Mean cpm	B/T%	βhCG mIU/mL
T	386282		
S1	103		0
S2	628	0.136	5
S3	2278	0.563	20
S4	7331	1.871	80
S5	26372	6.800	300
S6	69725	18.024	1000
C	2831	0.706	25.65

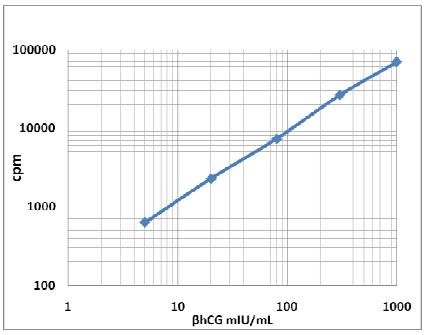

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples!*)

Characterization of assay

Typical assay parameters

NSB/T < 0.3 %

Sensitivity

For the analytical sensitivity 0.651 mIU/ml has been obtained by assaying 20 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Hook effect

There is no high dose " hook effect " up to a ßhCG concentration of 140000 mIU/ml .

Specificity

The monoclonal antibodies used in this IRMA kit are specific for ßHCG. No cross reactivity with hFSH, hLH and hTSH can be detected in normal physiological concentrations.

Precision

4 patient samples were assayed in 15 replicates to determine intra-assay precision. Values obtained are shown below.

Sample	Number of replicates	Mean value	SD	CV %
1	15	59.69	1.97	3.3
2	15	84.96	1.95	2.3
3	15	149.1	3.58	2.4
4	15	304.0	6.69	2.2

Reproducibility

To determine inter-assay precision 4 patient samples were measured in duplicates in 15 independent assays by 2 operators using different kit batches. Values obtained are shown below.

Sample	Number of runs	Mean value	SD	CV %
1	15	151.1	7.71	5.1
2	15	264.8	4.77	1.8
3	15	358.6	9.68	2.7
4	15	512.6	13.8	2.7

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of ßhCG. The average per cent recovery for 4 serum pooles spiked with ßhCG at 5 levels was: 101.99 ± 4.1 (mean ± SD).

Dilution test (linearity)

3 samples were measured in a series of dilution with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

y = 0.9787**x** + 1.8981 R = 0,9995 n =15

Expected Values

Healthy adult men and pre-menopause women (expect pregnant women):< 5 mIU/ml
Post-menopause women: < 10 mIU/ml
At 16-week gestation:35000mIU/ml (1MoM)
It is recommended that each laboratory determine a reference range for its own patient population.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg).
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 60 mg.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	DIL	Serum diluent
	Manufacturer	8°C 2	Temperature limitation Store between 2-8°C
REF	Catalogue number		
	Radioactive Material		
CE			

Website: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

IZOTOP
INSTITUTE OF ISOTOPES CO. LTD.

1535 Budapest. Pf.: 851.
Tel.: (36-1)392-2577
Fax: (36-1)395-9247

01449

Updated: 31.01.2014