Décembre 2021 – Modèle 18

Cisbio Bioassays
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hCG

REF ***OCPM03-HCG***

Σ 100

IVD

CE

2°C – 8°C

Trousse pour la détermination radioimmunologique de la gonadotrophine chorionique humaine (hCG)

Pour diagnostic In Vitro

La trousse contient :

Tubes revêtus	2 x 50
Traceur ≤ **300 kBq**	1 x 22 mL
Calibrateurs 0 – 7	8 x qsp 0,5 mL
Sérum de contrôle	1 x qsp 0,5 mL
Tampon d'incubation	1 x 50 mL
Réactif de lavage	2 x 3 comprimés
Sachet plastique	1
Notice d'utilisation	1

Attention : Certains réactifs contiennent de l'azoture de sodium

Kit for the radioimmunological determination of human chorionic gonadotropin (hCG)

For In Vitro diagnostic use

Kit content :

Coated tubes	2 x 50
Tracer ≤ **300 kBq**	1 x 22 mL
Calibrators 0 – 7	8 x qs 0.5 mL
Control serum	1 x qs 0.5 mL
Assay buffer	1 x 50 mL
Wash reagent	2 x 3 tablets
Plastic bag	1
Instruction for use	1

Warning : Some reagents contain sodium azide

Immunoradiometrischer Test zur quantitativen Bestimmung von freiem humanem Choriongonadotropin (hCG)

Zur In Vitro Diagnostik

Inhalt des Kits :

Teströhrchen beschichtet	2 x 50
Tracer ≤ **300 kBq**	1 x 22 mL
Kalibratoren 0 – 7	8 x qs 0,5 mL
Kontrollserum	1 x qs 0,5 mL
Inkubationspuffer	1 x 50
Waschreagenz	2 x 3 Tabletten
Plastikbeutel	1
Gebrauchsinformation	1

Achtung : Einige Reagenzien enthalten Natriumazid

Kit per il dosaggio radioimmunologico della gonadotropina corionica umana (hCG)

Per uso diagnostico In Vitro

Contenuto del kit :

Provette coattate	2 x 50
Tracciante ≤ **300 kBq**	1 x 22 mL
Calibratori 0 – 7	8 x q.b a 0,5 mL
Siero di controllo	1 x q.b a 0,5 mL
Tampone di incubazione	1 x 50
Reagente di lavaggio	2 x 3 compresse
Sacchetto di plastica	1
Istruzioni per l'uso	1

Attenzione : Alcuni reagenti contengono sodio azide

	FRA	ENG	DEU	ITA	POL
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Wyjaśnienie symboli**
CE	Conforme aux normes européennes	European conformity	CE-Konformitäts-kennzeichnung	Conformita europea	Zgodne z normami europejskimi
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Graniczna temperatura przechowywania
LOT	N° de lot	Batch code	Chargencode	codice lotto	Numer partii
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Zużyć do
	Consulter la notice d'utilisation	Consult operating instructions	Das Handbuch zu Rate ziehen	consultare le istruzioni per l'USO	Patrz dołączona ulotka
IVD	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Diagnostyka In Vitro
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Wyprodukowane przez
REF	Référence	Catalogue number	Katalog Nr.	N. catalogo	Wzorzec
	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Liczba probówek
CT	Tubes revetus	Coated tubes	Beschichtete Rohre	tubi rivestiti	rury w
TRACER	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Tracciante radioattivo	Znacznik radioaktywny
CAL	Calibrateur	Calibrator	Kalibrator	Calibratore	Kalibrator
CONTROL	Contrôle	Control	Kontrolle	Controllo	Kontrola
BUF	Tampon incubation	Incubation buffer	Inkubationspuffer	tampone di incubazione	bufor do inkubacji
WASH	Solution de lavage	Wash solution	Waschlotion	Soluzione di lavaggio e	Roztwór płuczący

MISE A JOUR / UPDATING

CE

OCPM03-HCG
Cisbio Bioassays – Décembre 2021 - Modèle 18

FRA

Modifications par rapport à la version précédente :
Changement de conditionnement des comprimés de lavage (blister de 3 au lieu de 5).

ENG

Changes from the previous version:
Change of packaging of wash solution tablets (blister of 3 tablets instead of 5).

DEU

Änderungen gegenüber der Vorgängerversion:
Packung der Waschpuffertabletten geändert (Blister mit 3 Tabletten statt 5)

ITA

Modifiche rispetto alla versione precedente:
Cambiamento del confezionamento delle compresse per la soluzione di lavaggio (blister da 3 compresse anziché 5)

POL

Zmiany w stosunku do poprzedniej wersji:
Zmiana opakowania zawierającego tabletki buforu płuczącego (blister zawierający 3 tabletki zamiast 5)

Kit for the radioimmunological determination of human chorionic gonadotropin (hCG) / Coated tube, ß-specific

Kit is intended for professional use.

The kit comprises:

1 vial of **^{125}I-anti-α-hCG** (monoclonal, mouse), < 300 kBq, 22 mL buffer with bovine albumin, rat serum, sodium azide and red dye.

2 x 50 **test tubes** coated with anti-β-hCG antibodies (monoclonal, mouse).

8 vials of hCG **calibrators,** freeze dried, qs 0.5 mL distilled water. Human serum and sodium azide, concentration in the nominal range of 0-1000 mIU hCG/mL*.

1 vial of hCG **control serum,** freeze dried, qs 0.5 mL distilled water. Human serum and sodium azide, concentration stated.

1 vial of **incubation buffer**, 50 mL of buffer, bovine albumine, nonspecific mouse immunoglobulins, sodium azide and blue dye.

1 **wash reagent**, 2 blisters of 3 tablets.

1 plastic bag.

1 instruction for use.

* The values shown above are the target values. The actual values of each calibrator and control are shown reagents labels.

The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

1. Introduction

Human chorionic gonadotropin (hCG) which consists of 237 amino acids and approximately 30% carbohydrates (molecular weight approximately 40.000) is made up, like the other glycoprotein hormones hLH, hFSH and hTSH, of two non-covalently bound subunits α and β. Only the complete intact hCG molecule exhibits the biological action. Structurally it comes closest to hLH which possesses 3 fewer amino acids in the α-chain and 30 fewer amino acids in the β-chain. The identity of several partial sequences results in similar binding characteristics at receptors and antibodies. The physiological activity of hCG too is similar to that of hLH ; at the beginning of pregnancy the function of the corpus luteum is stimulated and the synthesis and secretion of the placental steroids influenced.

2. Clinical results with RIA- gnost® hCG

2.1. Clinical significance of the quantitative hCG determination

hCG is normally not detectable in the serum of men and non-pregnant women. During pregnancy, however, the hCG-values increase rapidly 1-2 weeks after conception (3-4 weeks after the last menstruation) and reach a maximum in the 2nd to 3rd month. During the 2nd and 3rd trimester the values drop markedly again, large individual differences being observable. After giving birth the hCG values again drop below the limit of detection at a half-life of 24-36 hours.
Values above the norm distinctly indicate multiple pregnancies, whilst too low hCG values are observed in non-intact pregnancies (e.g. ectopic pregnancy, threatened abortions). Pathologically increased hCG concentrations occur in the presence of trophoblastic tumours and also in a large number of non-trophoblastic tumours. Thus the hCG test is suitable for clinical application in gynaecology and oncology for the detection and control of the course of pregnancies and tumours.

2.2. Normal values

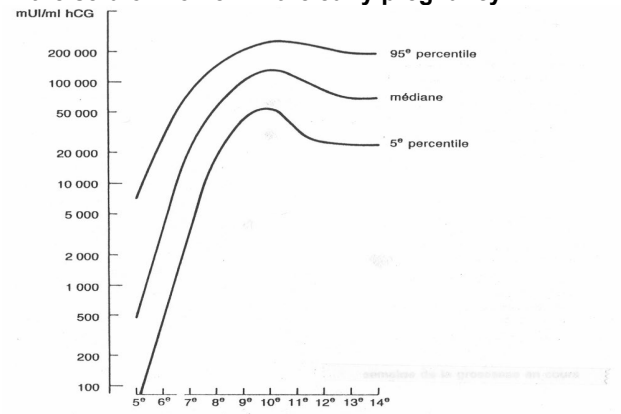
2.2.1. Tumour diagnosis

The upper limits of the normal region have been determined by n=165 sera of healthy men and n=96 sera of healthy non-pregnant women using the 95th percentile. For premenopausal women and men therefrom results an upper limit of 5 mIU hCG/mL, whereas this value is increased to 10 mIU hCG/mL (n = 157) for postmenopausal women.
Serum values above these limits can be considered as a hint for the presence of a tumour. The tumour marker hCG is in particular suitable for the follow-up of patients with hCG-producing tumours.

2.2.2. Pregnancies

The establishment of the expected values of RIA gnost®-hCG has been carried out by the measurement of 329 sera of normal pregnancies between the 5th and 14th current week of gestation. For the definition of the normal region, the median (50th percentile), the 5th and 95th percentile have been calculated (c.f. figure 1 and table 1)

Fig.1 : Normal region of the hCG-concentration in the sera of women in the early pregnancy



Current weeks of gestation

Table 1 : Normal region of the hCG-concentration in the sera of women in the pregnancy.

Current weeks of gestation	Number n	Median (mIU hCG/mL)	Range (mIU hCG/mL)
Non-pregnant	96	0	< 10
5	42	480	60 - 7 100
5-6	105	2 300	110 - 16 200
6-7	115	8 400	530 - 62 200
7-8	81	30 700	3 000 - 130 000
8-9	69	82 700	17 800 - 207 400
9-10	69	121 500	30 600 - 254 600
10-11	53	121 600	34 400 - 244 800
11-12	46	97 300	29 100 - 213 300
12-13	35	67 650	25 900 - 208 400
13-14	28	71 100	30 600 - 189 000
2nd trimester	92	27 500	10 100 - 92 200
3rd trimester	34	15 200	3 360 - 43 500

3. Principle of measurement and characteristic data of the RIA-gnost®hCG

3.1. Principle

RIA-gnost® hCG enables the **in-vitro determination** of intact, biologically active chorionic gonadotropin (hCG) in serum by the principle of a sandwich assay. Monoclonal mouse anti-β-hCG antibodies are the basis ; these are immobilized on the internal wall of the lower part of the incubation tubes. After adding the calibrator or serum samples the hCG is bound to the tube wall via the solid phase antibody during the first incubation step. After removal of serum and excess antigen the hCG that is present is quantified in the second incubation step by the binding of a monoclonal mouse anti-α-hCG (^{125}I-labelled) antibody. As during the normal pregnancy hCG values to be expected exceed 200 000 U/mL, and as a higher sensitivity is demanded for the early pregnancy test and tumour diagnosis, a great measuring range (0-1000 mIU hCG/mL) has been combined with a high sensitivity (< 1 mIU hCG/mL;CAL 0 variance). The signal/concentration relationship is definite over the entire measuring range (no “high-dose-hook-effect”).
RIA-gnost hCG has been **standardized** against the1st IRP (WHO 75/537) in mIU/mL. Since only the intact natural hCG is measured in the case of the hCG-IRMA, identical values are obtained when calibrated against the 2nd IS (WHO 61/6 of 1963) contaminated with subunits.

3.2. Imprecision

This was evaluated with 3 samples assayed 10 times in the same series and in 22 different series.

Within-run			Between-run		
Samples	**Mean (mIU/mL)**	**CV (%)**	**Samples**	**Mean (mIU/mL)**	**CV (%)**
1	12.9	2.4	4	11.7	11.4
2	50.0	2.8	5	22.0	8.7
3	90.0	1.8	6	149	3.8

4. Working instructions

4.1. Equipment required

Precision micropipettes or similar, with disposable tips, permitting the dispensing of 20 µL, 50 µL, 100 and 200 µL. Their calibration should be checked regularly.
1 mL Dispensette. Measuring cylinders. Horizontal shaker. Gamma scintillation counter

4.2. Preparation of the reagents

The reagents which have been stored at 2-8°C are ready for use at 18-25°C. The lyophilized reagent (calibrators and control) have to be dissolved in 0.5 mL of distilled water. After complete dissolution, they could be used within 4 hours at room temperature (18-25°C), stored 15 days at 2-8°C or stored frozen for 45 days. They could be frozen and thawed once. 5 tablets of the wash buffer have to be dissolved in 500 mL distilled water (the remaining tablet is provided if needed).

General Hints

Reagents from more than one kit may be pooled in order to perform larger assays if they are from the same batch. Unused coated tubes are stored in the resealed package at 2-8°C. Unused antibodies coated tubes after packaging opening must be stored in the plastic bag supplied with the kit.

4.3. Preparation of the samples

The assay is performed directly on serum or plasma. The serum or plasma can be used immediately or stored for up to 24 hours at maximum 2-8°C before the assay. If stored for a longer period the temperature must be –20°C. After thawing, shake well the samples. Sera with hCG values out of the measuring range have to be diluted. The kit contains an assay buffer which makes it possible, in addition to its use as the incubation medium, to prepare the dilutions.

If the assay buffer is not sufficient the first dilution in the case of highly elevated values should be carried out by using the wash buffer.
For the pregnancy follow-up dilutions of 1:20 and 1:200 are recommended.
When performing the dilution steps take care to ensure good mixing.
Urine samples too can be used for the qualitative process.

4.4. Warnings and Precautions
Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti HIV 2, anti-HCV antibodies and the HBs antigen. However, as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

4.5 Performance of the hCG determination
One must differentiate between the qualitative assay for the early pregnancy test and the quantitative assay for control of the course of pregnancy and tumour diagnosis.

4.5.1 Qualitative assay
1. Number sufficient coated test tubes as stated in table 2 (a negative control = CAL 0, a positive control = control serum, a reference calibrator = CAL 3, unknown samples).
2. Pipet 100 µL of each calibrator and patient sample on the bottom of the appropriately labelled tubes. Use a new pipette tip for each sample.
3. In each tube dispense 100 µL assay buffer solution.
4. Subsequently shake the tubes on a horizontal shaker for 10-30 minutes at 18-25°C.
5. Then pipette 1 mL washing buffer by means of a dispenser into each tube, decant (aspirate). Wash again with 1 mL washing buffer.
6. Dispense 200 µL tracer solution on the bottom of each incubation tube.
7. Shake for 10-60 minutes as described in 4.
8. Washing as described in 5.
9. Measure the tubes for 1 minute in the gamma scintillation counter.

Table 2 : Performance of the qualitative hCG assay

	Negative control (CAL 0) (µL)		**Positive control (control serum) (µL)**		**Reference calibrator (CAL 3) (µL)**		**Samples (µL)**				
Number of the test tube ►	**CAL 0**		**C**		**CAL 3**		**1**		**2**		**etc**
Negative control (CAL 0)	100	100									
Positive control (Control serum)			100	100							
Reference calibrator (CAL 3)					100	100					
Sample 1							100	100			
Sample 2, etc.									100	100	etc.
Assay buffer	◄----------100 µL ----------►										
	Shake for 10-30 minutes										
Wash buffer	◄----------1 mL----------►										
	Decant (aspirate) ; wash again with 1 mL										
Anti-α-hCG-tracer	◄----------200 µL----------►										
	Shake for 10-60 minutes										
Wash buffer	◄----------1 mL ----------►										
	Decant (aspirate) ; wash again with 1 mL										
	Measure the tubes										

Evaluation of the results of the qualitative process

1. Calculate the averages of the cpm of the calibration samples (neg./pos. control, reference calibrator) and serum samples all measured in duplicate.
2. The cpm of the negative control (CAL 0) must be significantly below the reference sample and the positive control (control serum) distinctly above the reference sample.
3. Samples whose cpm are above the reference calibrator are to be considered positive. If cpm are found in the borderline region a further sample should be taken 48 hours later in order to confirm the result (see table 3).
   Samples whose cpm are below the reference calibrator are to be considered negative.

Table 3 : Example for the evaluation of the qualitative assay

	Average (cpm)	Results
Reference calibrator (CAL 3)	1 128	-
Negative control (CAL 0)	120	-
Positive control (control serum)	2 584	-
sample 1	167	negative
sample 2	2 192	positive
sample 3	140	negative
sample 4	1 070	border line

4.5.2. Quantitative assay

1. Number sufficient coated test tubes as stated in table 4 (CAL 0- CAL 7; control serum, 41 Serum samples). In each case it is advisable to assay duplicate calibrators, control and samples
2. Place the calibrators and patient samples (serum of buffer dilution [blue] in 100 µL aliquots on the bottom of the appropriately labelled tubes. Use a new pipette tip for each sample.
3. In each tube place 100 µL assay buffer solution.
4. Subsequently shake the tubes on a horizontal shaker for 30 minutes at 18-25°C.
5. Then pipette 1 mL washing buffer by means of a dispenser into each tube, decant (aspirate). Wash again with 1 mL washing buffer.
6. Dispense 200 µL tracer solution on the bottom of each incubation tube.
7. Shake for 60 minutes as described in 4.
8. Washing as described in 5.
9. Measure the tubes for 1 minute in the gamma scintillation counter.

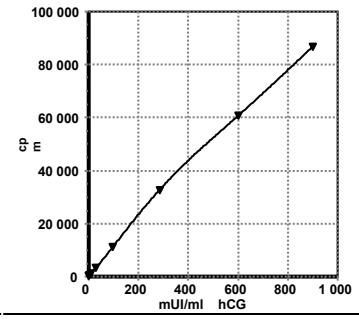
Table 4 : Performance of the quantitative hCG assay

	Calibrators (µL)								Control serum (µL)	Samples (µL)		
Number of the test tube	**CAL 0**	**CAL 1**	**CAL 2**	**CAL 3**	**CAL 4**	**CAL 5**	**CAL 6**	**CAL 7**	**C**	**1**	**2**	**etc**
Calibrators CAL 0	100/100											
CAL 1		100/100										
CAL 2			100/100									
CAL 3				100/100								
CAL 4					100/100							
CAL 5						100/100						
CAL 6							100/100					
CAL 7								100/100				
Control serum HCG-concentration declared									100/100			
Samples										100	100	etc.
Assay buffer	◄----------100 µL ----------►											
	Shake for 30 minutes											
Wash buffer	◄----------1 mL ----------►											
	Decant (aspirate) ; wash again with 1 ml											
Anti-α-hCG tracer	◄----------200 µL ----------►											
	Shake for 60 minutes											
Wash buffer	◄----------1 mL ----------►											
	Decant (aspirate) ; wash again with 1 mL											
	Measure the tubes											

Evaluation of the results of the quantitative assay
For each group of tubes, calculate the mean counts.
Draw up the standard curve by plotting the calibrators' cpm against their concentrations.
Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.
The spline mathematical fitting model is recommended for calibration curve. Other fitting model may give slightly different results.

Fig.2 : Example of a calibrator curve

Group of tubes	Mean cpm	Concentration mUI/mL
Calibrator 0	176	0
Calibrator 1	543	3.6
Calibrator 2	1246	9.0
Calibrator 3	3346	28.5
Calibrator 4	11168	97.0
Calibrator 5	32766	285.0
Calibrator 6	69704	600.0
Calibrator 7	86786	900.0
Control	14017	122



4.6 Interference

No interference with bilirubin, haemoglobin, and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L, and 20 g/L, has been observed.
No biotin interference measured up to 1200 ng/mL was observed.
The immunoassay is protected against any human anti-mouse antibody (HAMA) interference by the addition of a protector (non-specific mouse immunoglobulins) to the incubation buffer. However, we cannot guarantee that this protection is exhaustive.

5. Radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate safety.
A summary of these is given below:
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up-to-date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone.
Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

BIBLIOGRAPHY :

Berger P, Sturgeon C, Bidart JM, Paus E, Gerth R, Niang M, Bristow A, Birken S, Stenman UH. The ISOBM TD-7 workshop on HCG and Related Molecules. Tumor Biol. 2002;23:1-38.

Braunstein GD, Rasor J, Adler D, Danzer H, Wade ME. Serum human chorionic gonadotropin levels throughout normal pregancy. Am J Obstet Gynecol. 1976;15:678-81.

Licht P, Cao H, Lei ZM, Rao CV, Merz WE. Novel self-regulation of human chorionic gonadotropin biosynthesis in term human placenta. Endocrinol. 1993;133:3014-25.

Lottersberger C, Hoermann R, Mann K, Schwarz S, Berger P. Tumor and Pregnancy Derived Isoforms of human chorionic gonadotropin. Biological and Diagnostic Relevance. Horm Res. 2003;59:125-134.

Ozturk M. Human chorionic gonadotropin, its free subunits and gestational trophoblastic disease. J Reprod Med. 1991;36:21-6.

Pierce JG, Parsons TF. Glycoproteins hormones : structure and function. Ann Rev Biochem. 1981;50:465-95.

Rice L, Berkowitz R, Lage J, Goldstein D, Bernstein M. Persistent gestational trophoblastic tumor after partial hydatidiform mole. Gynecol Oncol. 1990;36:358-62.

Saller B, Clara R, Spottl G, Siddle K, Mann K. Testicular cancer secretes intact human choriogonadotropin (hCG) and its free beta-subunit : evidence that hCG (+hCG-beta) assays are the most reliable in diagnosis and follow-up. Clin Chem.1990;36:234-9.

Storring PL, Gaines-Das RE, Bangham DR. International reference preparation of human chorionic gonadotropin for immunoassay. J Endocrinol. 1980;84:295-310.