Juin 2018 – Modèle 018

Cisbio Bioassays
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THYROGLOBULINE IRMA

REF *THYRO* Σ 100 IVD CE 2°C 8°C

Trousse pour le dosage immunoradiométrique de la thyroglobuline humaine en sérum ou plasma **Pour diagnostic In Vitro**		**kit for an immunoradiometric assay of serum or plasma human thyroglobulin** **For In Vitro diagnostic use**		**Immunoradiometrischer Test zur Bestimmung von Human-Thyreoglobulin im Serum oder Plasma** **Zur In Vitro Diagnostik**	
La trousse contient :		Kit content :		Inhalt des Kits :	
Tubes revêtus	2 x 50	Coated tubes	2 x 50	Teströhrchen beschichtet	2 x 50
Traceur ≤ **481 kBq**	1 x 42 mL	Tracer ≤ **481 kBq**	1 x 42 mL	Tracer ≤ **481 kBq**	1 x 42 mL
Solution de lavage	1 x 25 mL	Washing solution	1 x 25 mL	Waschlösung	1 x 25 mL
Solution de tampon	1 x 35 mL	Buffer solution (R4)	1 x 35 mL	Pufferlösung (R4)	1 x 35 mL
Calibrateurs (CAL1 - CAL7)	7 x 1 mL	Calibartors (CAL1 - CAL7)	7 x 1 mL	Kalibratoren (CAL1 - CAL7)	7 x 1 mL
Sérum de contrôle (C1)	1 x qsp 1 mL	Control sera (C1)	1 x qs 1 mL	Kontrolle (C1)	1 x qs 1 mL
Sérum de contrôle (C2)	3 x qsp 1 mL	Control sera (C2)	3 x qs 1 mL	Kontrolle (C2)	3 x qs 1 mL
Sachet plastique	1	Plastic bag	1	Plastikbeutel	1
Notice d'utilisation	1	Instruction for use	1	Arbeitsanleitung	1
Attention : Certains réactifs contiennent de l'azoture de sodium		**Warning :** Some reagents contain sodium azide		**Achtung :** Einige Reagenzien enthalten Natriumazid	

Kit per il dosaggio immunoradiometrico della tireoglobulina umana in siero o plasma **Per uso diagnostico In Vitro**		**Equipo para la determinación inmunorradiométrica de la tiroglobulina humano en suero o plasma** **Para diagnóstico in vitro**		**Τυποποιημένη συσκευασία για τον ραδιοανοσολογικό προσδιορισμό της ανθρώπινης θυρεοσφαιρίνης του ορού ή του πλάσματος** **Για διαγνωστική χρήση in vitro**	
Contenuto del kit:		Contenido del equipo:		Περιεχόμενα της τυποποιημένης συσκευασίας	
Provette coattate	2 x 50	Tubos recubiertos	2 x 50	Επικαλυμμένοι σωλήνες	2 x 50
Tracciante ≤ **481 kBq**	1 x 42 mL	Trazador ≤ **481 kBq**	1 x 42 mL	Ιχνηθέτης ≤ **481 kBq**	1 x 42 mL
Soluzione di lavaggio	1 x 25 mL	Reactivo de lavado	1 x 25 mL	Διάλυμα έκπλυσης	1 x 25 mL
Soluzione tampone	1 x 35 mL	Reactivo de tampón	1 x 35 mL	Ρυθμιστικό διάλυμα	1 x 35 mL
Calibratori (CAL1 - CAL7)	7 x 1 mL	Calibradores (CAL1 - CAL7)	7 x 1 mL	Πρότυπα (CAL1 - CAL7)	7 x 1 mL
Siero di controllo (C1)	1 x q.ba 1 mL	Control (C1)	1 x csp 1 mL	Οροί μάρτυρες (C1)	1 x qs 1 mL
Siero di controllo (C2)	3 x q.ba 1 mL	Control (C2)	3 x csp 1 mL	Οροί μάρτυρες(C2)	3 x qs 1 mL
Sacchetto di plastica	1	Bolsa de plástico	1	Πλαστική θήκη	1
Istruzioni per l'uso	1	Instrucciones de empleo	1	Οδηγίες χρήσεως	1
Attenzione: Alcuni reagenti contengono sodio azide		**Precauciones:** Algunos reactivos contienen azida sódica		**Προσοχή :** Ορισμένα αντιδραστήρια περιέχουν αζίδιο νατρίου	

	FRA	ENG	DEU	ITA	SPA	ELL	POR	SWE	POL	SRB
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Significado de los simbolos**	**Επεξήγηση των συμβόλων που**	**Significadodos simbolos**	**Symbol förklaring**	**Wyjaśnienie symboli**	**Objašnjenje simbola**
	Conforme aux normes européennes	European conformity	CE-Konformitäts-kennzeichnung	Conformita europea	Conformidad europea	European conformity	Conformidad com as normas europeias	Förenlig med europeiska normer	Zgodne z normami europejskimi	Evropska usaglašenost
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Limites de temperatura de almacenamiento	Περιορισμός θερμοκρασίας φύλαξης	Limite da temperatura de armazenagem	T°-gräns vid förvaring	Graniczna temperatura przechowywania	Ograničenje temperature za čuvanje
	N° de lot	Batch code	Chargencode	codice lotto	Código de lote	Κωδικός παρτίδας	Lote	Lotnr.	Numer partii	Šifra serije
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Consumir antes de	Ημερομ. λήξης	Utilizado por	Används senast	Zużyć do	Upotrebiti do
	Consulter la notice d'utilisation	Consult operating instructions	Das Handbuch zu Rate ziehen	consultare le istruzioni per l'USO	Consultar las instrucciones de manejo o funcionamiento	Ανατρέξτε στις οδηγίες λειτουργίας	Consulte o manual de operações	Läs bruksanvisningen	Patrz dołączona ulotka	Pogledajte uputstvo za upotrebu
	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Dispositivo de diagnóstico In Vitro	διαγνωστική συσκευή In Vitro	Dispositivo de diagnostico In Vitro	*In vitro*-diagnos	Diagnostyka In Vitro	Uređaj za dijagnostiku *in vitro*
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Fabricado por	Κατασκευάζεται από την	Fabricado por	Tillverkad av	Wyprodukowane przez	Proizveo
	Référence	Catalogue number	Katalog Nr.	N. catalogo	Número de catálogo	Αριθμός καταλόγου	Número do catalogo	Referens	Wzorzec	Kataloški broj
	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Número de determinaciones	Αριθμός προσδιορισμών	Número de determinações	Antal rör	Liczba probówek	Broj određivanja
	Tubes revêtus	Coated tubes	beschichtete Röhrchen	Provette coattate	Tubos recubiertos	Επιστρωμένα σωληνάρια	Tubos adsorvidos	Belagda rör	Probówki powlekane	Obložene epruvete
	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Tracciante radioattivo	Trazador radiactivo	Ραδιενεργός ιχνηθέτης	Marcador radioativo	Radioaktiv tracer	Znacznik radioaktywny	Radioaktivni indikator
	Calibrateur	Calibrator	Kalibrator	Calibratore	Calibrador	Βαθμονομητής	Calibrador	Kalibrator	Kalibrator	Kalibrator
	Contrôle	Control	Kontrolle	Controllo	Control	Ορός ελέγχου	Controle	Kontroll	Kontrola	Kontrola
WASH	Solution de lavage à diluer x fois	Wash solution to be diluted n-fold	Waschlotion zum x-maligen Verdünnen	Soluzione di lavaggio da diluire n volte	Solución de lavado que debe diluirse n veces	Διάλυμα πλύσης που πρέπει να αραιωθεί n φορές	Solução de lavagem a ser diluída n X	Tvättlösning utspädes x gånger	Roztwór płuczący do rozcieńczania x razy	Rastvor za pranje treba razblažiti n-puta

MISE A JOUR / UPDATING

CE

THYRO

Cisbio Bioassays - Juin 2018 - Modèle 018

FRA **Modifications par rapport à la version précédente :**
Modification code langue Serbe.

ENG **Changes from the previous version:**
Modification Serbian language code.

DEU **Änderungen gegenüber der Vorgängerversion:**
Änderung serbischer Sprachcode.

ITA **Modifiche rispetto alla versione precedente:**
Modifica codice lingua serba.

SPA **Cambios desde la versión anterior:**
Modificación del código de idioma serbio.

ELL **Αλλαγές από την προηγούμενη έκδοση:**
Τροποποίηση κώδικα σερβικής γλώσσας.

POR **Alterações em relação à versão anterior:**
Modificação do código de idioma sérvio.

SWE **Ändringar från den föregående versionen:**
Ändring serbisk språkkod.

POL **Zmiany w stosunku do poprzedniej wersji:**
Modyfikacja kodu języka serbskiego.

SRB **Promene od prethodne verzije:**
Izmjena kod srpske jezika.

ENG

CE

THYRO

Cisbio Bioassays - June 2018 - Model 018

1. NAME AND INTENDED USE

THYRO is an immunoradiometric test for the assay of serum or plasma human thyroglobulin.
The kit is intended for professional use.

2. INTRODUCTION

The THYRO kit is an immunoradiometric test for the assay of human thyroglobulin.
Thyroglobulin, an iodinated glycoprotein with a molecular weight of 660,000, is the principal constituent of vesicular colloid. It is synthesized exclusively by the thyroid cell for which it therefore constitutes a specific marker. Thyroglobulin plays an essential role in the biosynthesis, storage and secretion of thyroid hormones T3 and T4.
Its assay is useful :

- In oncology : to monitor the course of differentiated thyroid carcinomas. After total resection of the thyroid, thyroglobulin constitutes an early reliable marker of the development of metastases.
- In benign thyroid disease : the assay of thyroglobulin can be used to follow the course of Graves'disease and to guide therapeutic withdrawal.
  The thyroglobulin levels are also increased in various forms of thyroiditis, especially in Hashimoto's chronic thyroiditis.
- In the differential diagnosis of thyroid and parathyroid cysts by simultaneous assay of thyroglobulin and parathormone.
- For the differential diagnosis of thyroid agenesis and ectopia : in neonatal hypothyroidism, the presence of thyroglobulin suggests the diagnosis of ectopia, while thyroglobulin is absent in agenesis.
- For the differential diagnosis of thyrotoxicosis. In amiodarone-induced thyrotoxicosis, the thyroglobulin levels are high, while in pseudo-thyrotoxicosis, the thyroglobulin levels are undetectable.

  Up until now, anti-thyroglobulin auto-antibodies present in the serum have affected the results of thyroglobulin assays.
  The THYRO kit uses monoclonal antibodies selected for their recognition of domains of thyroglobulin not recognised by the patient' s auto-antibodies, thereby allowing a reliable assay of thyroglobulin even in the presence of autoantibodies.

3. PRINCIPLE

The THYRO kit uses an immunoradiometric technique with the following characteristics :

- A mixture of four monoclonal anti-thyroglobulin antibodies, selected according to well defined criteria of specificity, avidity and complementarity, is coated onto the walls of the tubes.
- A fifth monoclonal antibody, iodine 125 labelled, recognizing a different epitope from those recognized by the antibodies bound to the tubes, is used as a tracer.
- These antibodies are directed against the epitopic zones not recognized by the majority of anti-thyroglobulin autoantibodies present in numerous thyroid diseases, thereby avoiding the systematic need for a spiked test.
- After incubation of the standards and the samples in the presence of the antibodies bound in excess to the tubes as well as with an excess of labelled antibodies, and elimination of the unbound fraction by washing, measurement of the activity bound to the tube allows calculation of the thyroglobulin concentration.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
COATED TUBES: ready for use. Monoclonal anti-thyroglobulin antibodies coated on the bottom of the tube.	CT	2 packs of 50 tubes	2-8°C until the expiry date. After packaging opening, unused antibodies coated tubes must be stored in the plastic bag, with the dessicant.
ANTI-THYROGLOBULIN ^{125}I: ready for use. Iodine 125 labelled monoclonal anti-thyroglobulin antibody diluted in Phosphate / non specific immunoglobulins / Tween /Casein buffer, pH 7.4 containing 0.1 % sodium azide, red coloured. The vial contains a maximum of 13 µCi, i.e. **481 kBq** of 125I anti-thyroglobulin antibody with a specific activity of 12 Ci/g, i.e. about 200,000 cpm for 400 µL (444 kBq/µg).	TRACER	1 vial of 42 mL	2-8°C until the expiry date.
CALIBRATORS (CAL1 – CAL7) : ready for use. Human thyroglobulin, diluted in Phosphate pH 7.4 buffer containing 0.1 % sodium azide, orange coloured. 0.2 – 1.5 – 5 - 15 – 50 – 200 - 500 ng/mL(*).	CAL	7 vials of 1 mL	2-8°C until the expiry date.
CONTROL SERUM (C1) : lyophilised (**). Human serum containing 0.1% sodium azide. Reconstitute each vial with 1 mL of distilled water.	CONTROL	1 vial qsp 1 mL	2-8°C until the expiry date. After reconstitution: the control serum stored at +4°C can be used for 5 days. Any fraction not used can be frozen and stored at – 20°C. Only freeze once. The reagents are stable under these conditions for 2 months.
CONTROL SERUM (C2) : lyophilised (**). Human serum containing 0.1% sodium azide. Reconstitute each vial with 1 mL of distilled water.	CONTROL	3 vials qsp 1 mL	2-8°C until the expiry date. After reconstitution : the control serum stored at +4°C can be used for 5 days. Any fraction not used can be frozen and stored at – 20°C. Only freeze once. The reagents are stable under these conditions for 2 months.
WASHING SOLUTION : liquid. Imidazole pH 7.4 buffer containing 0,1 % sodium azide and tween 20. This solution must be diluted to 1/40 with distilled water.	WASH	1 vial of 25 mL	2-8°C until the expiry date.

BUFFER SOLUTION : ready for use. Phosphate pH 7.4 buffer containing 0.1 % sodium azide, orange coloured. Any dilutions of the sera must be performed with the buffer solution.	**BUF**	1 vial of 35 mL	2-8°C until the expiry date.
PLASTIC BAG		1	

(*) The values shown above are target values : they are shown on its label. The calibrators are calibrated against the CRM 457 (human thyroglobulin reference material).
(**) The acceptance range true values are printed on the vial label.

5. PRECAUTIONS FOR USE

5.1. Safety measures

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.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
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.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by people 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 security.
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.

5.3. Handling precautions

Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbic contamination of the reagents or of the water.
Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The test is performed on serum, or on plasma.
When the assay is performed on citrate samples, the results are 10% less than serum results. Citrate plasma = 0.86 x serum - 0.0075
$r^2 = 0.998$
The assay can be performed on samples stored at +2/+8°C for up to 5 days.
Beyond this time, store the serum samples at -20°C.
Avoid successive freezing and thawing.
Haemolysed or hyperlipemic samples should not be used.

Dilution

Should elevated thyroglobulin levels be suspected, dilution is performed with the buffer solution found in the kit.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar with disposable tips, capable of dispensing 100 µL, 300 µL and 2 mL. Their calibration should be checked regularly. Graduate cylinder 1 L). Distilled or deionised water. Vortex-type mixer. Circular horizontal shaker. Parafilm® paper. Aspiration system. Gamma scintillation counter calibrated for 125 iodine measurement.

7.2 Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use.
Dispensing of the reagents into the tubes is also carried out at room temperature.

The assay requires the following groups of tubes :
T group, for the total activity determination,
Calibrator groups to establish the standard curve,

Control group for the control,
Sx groups for the samples to be assayed.

- It is recommended to perform the assay in duplicate for calibrators, control and samples.
- The preparation of the standard curve and the assay of the samples must be performed simultaneously

a) Standard protocol

Observe the order in which reagents are to be added :

- Dispense 100 µL of calibrators, control serum or samples into the corresponding groups of tubes.
- Add 300 µL of the buffer solution into each tube, except tubes T.
- Cover the tubes with Parafilm® plastic film.
- Incubate 3 hours **with agitation (400rpm) at room temperature (18-25°C).**
- Wash the coated tubes as follows :
  Aspirate the contents of the tubes as completely as possible.
  Add 2,0 mL of washing solution to each tube.
  Aspirate.
  Repeat the process once.
  Leave the tubes to stand 2 minutes or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.
- Dispense 400 µL of tracer ^{125}I into each tubes.
- Cover the tubes with Parafilm® plastic film.
- Incubate overnight 16-20 hours **at room temperature (18-25°C) without agitation**.
- Wash the coated tubes as follows :
  Aspirate the contents of the tubes as completely as possible.
  Add 2,0 mL of washing solution to each tube.
  Aspirate.
  Repeat the process once.
  Leave the tubes to stand 2 minutes or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

  To obtain reliable and reproducible results, the different washing steps have to be correctly performed. The addition of the washing solution must be carried out with an efficient speed in order to create turbulences into the tubes.
- Measure the remaining radioactivity bound to the tube with a gamma scintillation counter over 1 minute.

STANDARD PROCEDURE (ASSAY FLOW CHART)
Order of addition and volume (in µL) of the reagents to be added to each tube

Tubes	**Calibrators (CAL1-CAL7) Controls (C1-C2)) Samples µL**	**Buffer solution µL**	Agitate - Incubate 3 hours **at 18-25°C under agitation 400rpm** Wash 2 times	**Tracer ^{125}I (R2) µL**	Incubate 16 – 20 h **at 18-25°C without agitation** Wash 2 times	Count
T	-	-		400		
Calibrators	100	300		400		
Controls Samples	100	300		400		

b) Rapid protocol

Observe the order in which reagents are to be added :

- Dispense 100 µL of calibrators, control serum or samples into the corresponding groups of tubes.
- Add 400 µL of tracer ^{125}I into each tube.
- Shake on a Vortex type shaker.
- Cover the tubes with Parafilm® plastic film.
- Incubate overnight (16-20 h) **at room temperature (18-25°C) without agitation**.
- Wash the coated tubes as follows :
  Aspirate the contents of the tubes as completely as possible.
  Add 2,0 mL of washing solution to each tube.
  Aspirate.
  Repeat the process once. Then, leave the tubes to stand 2 minutes or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

  To obtain reliable and reproducible results, the different washing steps have to be correctly performed. The addition of the washing solution must be carried out with an efficient speed in order to create turbulences into the tubes.
- Measure the remaining radioactivity bound to the tube with a gamma scintillation counter over 1 minute.

RAPID PROCEDURE (ASSAY FLOW CHART)

Order of addition and volume (in µl) of the reagents to be added to each tube

Tubes	Calibrators (CAL1-CAL7) Controls (C1 – C2) Samples µL	Tracer ^{125}I (R2) µL		
T	-	400	Agitate - Incubate 16 – 20 h **at 18-25°C** **(without agitation)** - Wash 2 times .	Count
Calibrators	100	400		
Controls Samples	100	400		

8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

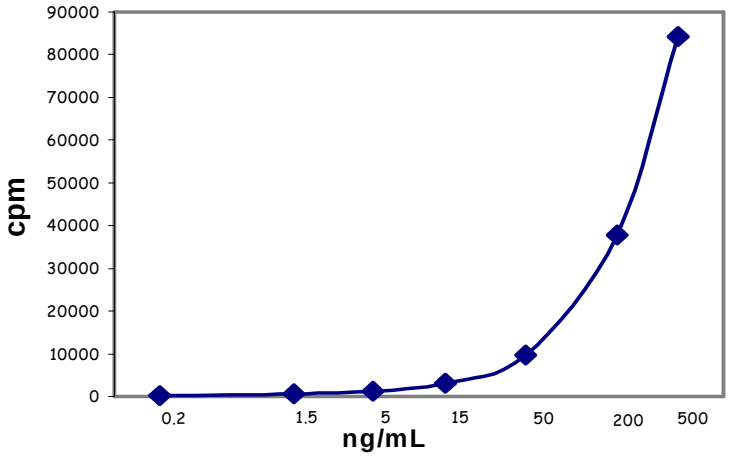
Preparation of the standard curve :

Calculate the mean count for each pair. If necessary , calculate the percentage binding B/T (%).
Draw up the calibration curve by plotting the calibrators'cpm (or B/T (%)) against their concentration.
The thyroglobulin concentration is determined by interpolation of the cpm or B/T(%).

The forced spline mathematical fitting model is recommended for calibration curve. Other fitting model may give slightly different results.

Typical calibrator curve (example only) : these data must under no circumstances be substituted for results obtained in the laboratory.

			CPM
Total activity			187089
CAL1	0.2	ng/mL	112
CAL2	1.5	ng/mL	529
CAL3	5	ng/mL	1207
CAL4	15	ng/mL	3130
CAL5	50	ng/mL	9635
CAL6	200	ng/mL	37848
CAL7	500	ng/mL	84289
C1	10.7	ng/mL	2708
C2	110	ng/mL	23854



10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.
Do not extrapolate sample values beyond the last calibrator. Dilute the concerned samples and re-assay.

11. EXPECTED VALUES

Each laboratory should establish its own range of normal values. The value given below are only indicative.
The values obtained in presumably healthy subjects of both sexes fall into the following concentration ranges (n = 149).

CONCENTRATION RANGE				NUMBER OF PATIENTS	%
0	-	5	ng/mL	11	7.4
5	-	10	ng/mL	37	25.0
10	-	20	ng/mL	61	41.0
20	-	30	ng/mL	20	13.0
30	-	50	ng/mL	15	10.0
>	-	50	ng/mL	5	3.4

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Precision of the test

The precision of the test is indicated by the within-run and between-run on assays reproductibility.

WITHIN-RUN

	REPRODUCTIBILITY			REPRODUCTIBILITY	
	Standard protocol n = 31			Rapid protocol n = 31	
SERUM	**X (ng/ml)**	**CV (%)**	**SERUM**	**X (ng/ml)**	**CV (%)**
A	0.73	7.0	F	0.69	6.6
B	5.2	2.6	G	3.9	3.7
C	39	2.4	H	25.6	4.5
D	105	1.7	I	88	1.6
E	320	1.8	J	214	1.5

BETWEEN-RUN

	REPRODUCTIBILITY			REPRODUCTIBILITY	
	Standard protocol n = 30			Rapid protocol n = 12	
SERUM	**X (ng/ml)**	**CV (%)**	**SERUM**	**X (ng/ml)**	**CV (%)**
A	0.95	14.6	F	0.92	12.1
B	3.9	4.9	G	3.4	8.6
C	37	4.6	H	33	3.5
D	101	6.2	I	89	6.5

12.2. Spiked test

SPIKED TEST

	Standard protocol			Rapid protocol	
SERUM	**Concentration (ng/ml)**	**Recovery (%)**	**SERUM**	**Concentration (ng/ml)**	**Recovery (%)**
Sample 1	157	101	Sample 5	137	99
Sample 2	207	104	Sample 6	180	105
Sample 3	204	108	Sample 7	169	101
Sample 4	121	103	Sample 8	107	101

Thyroglobulin assays can be influenced by the presence of anti-TG antibodies or other non-specific factors present in the patient's serum; it is therefore necessary to test the influence of this type of interference on serum by performing an overload test.

The overload test is performed as follows:
- Control C2: test portion = 100 µL
- Sample: test portion = 100 µL
- Overload test: test portion = 50 µL C2 + 50 µl sample
- Calculation of recovery percentage:

$$\frac{\text{Overload test}}{\left[\frac{\text{C2} + \text{sample}}{2}\right]} \text{x}\,100$$

Example:
where 48 ng/mL is the C2 concentration measured.
where 5 ng/mL is the sample concentration measured.

where 26 ng/mL is the concentration measured during the overload test. Recovery percentage = $\frac{26}{\left[\frac{48+5}{2}\right]} \text{x}\,100$ = 98.1%

12.3. Dilution test
Four samples have been diluted with the buffer solution.

Standard protocol			Rapid protocol		
Dilution factor	**Observed value (ng/ml)**	**Recovery (%)**	**Dilution factor**	**Observed value (ng/ml)**	**Recovery (%)**
Neat	92		Neat	85	
1/2	100	109	1/2	92	108
1/4	103	112	1/4	99	116
1/8	100	109	1/8	90	106
Neat	205		Neat	212	
1/2	229	112	1/2	214	101
1/4	236	115	1/4	222	105
1/8	243	119	1/8	236	111
1/16	239	117	1/16	217	102
Neat	337		Neat	299	
1/2	332	99	1/2	314	105
1/4	352	104	1/4	324	108
1/8	361	107	1/8	341	114
1/16	373	111	1/16	361	121
Neat	423		Neat	407	
1/2	418	99	1/2	436	107
1/4	432	102	1/4	430	106
1/8	467	110	1/8	446	110
1/16	492	116	1/16	488	120

12.4. Hook-effect

Thyroglobulin concentrations up to 28 000 ng/mL give a higher signal than the last standard, using the rapid procedure (one-step). However few samples with levels >8,000 ng/mL are usually encountered. With the standard procedure (two-steps) the hook effect is increased to greater than 800.000 ng/mL.

12.5. Detection limit

The detection limit measured by analytical method is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 0.2 ng/mL.
The functional sensitivity is defined as being the measured concentration by imprecision profile for a CV equal to 20 %. It has been assessed as being 0.7 ng/mL.

12.6. Measuring range

0.7 – 500 ng/mL

12.7. Interferences

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.
The immunoassay is protected against potential interferences like human anti - mouse antibodies (HAMA) by adding a protection in the tracer (non specific mice immunoglobulins).
Nevertheless , it cannot be guaranteed that there will never be detection of "
false positive " or " false negative " due to the presence of interferences like heterophilic antibodies , anti-avidin antibodies, rhumatoid factor , etc.. , in the patient samples.

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