

Tg-S

BL-03-CT

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IMMUNORADIOMETRIC ASSAY FOR THE QUANTITATIVE DETERMINATION OF THE HUMAN THYROGLOBULIN IN HUMAN SERUM AND PLASMA SENSITIVE METHOD

100 Determinations

FOR IN VITRO DIAGNOSTIC USE ONLY

1. CLINICAL APPLICATIONS

Thyroglobulin (**hTg**) is the major component of colloid in thyroid follicles. hTg is a glycoprotein of 660 kda. hTg being specific of the thyroid cell, the determination of its blood concentration is thus of great significance for the diagnosis of thyroid diseases.

In areas of endemic goiter, a majority of affected persons had substantially higher thyroglobulin levels than normal controls. However elevated thyroglobulin levels are also observed among patients with sporadic goiter in area where iodine intake is substantially higher. In both situations, these elevated values are correlated with the development of the goiter itself.

During the initial phase of subacute thyroiditis, the thyroglobulin levels are markedly elevated. Persistent high hTg levels at the end of a cure by antithyroid drug are considered predictive for a relapse of hyperthyroidism at the withdrawal of therapy. hTg determination is thus part of the monitoring of Graves' disease and its management.

2. PRINCIPLE OF THE ASSAY

This test kit is an immunoradiometric assay (IRMA) based on coated-tubes with monoclonal antibodies directed against distinct epitopes of the molecule of TG.

Three capture antibodies are coated on the inner wall of tubes. TG of the calibrators or of the samples is captured by these antibodies.

Addition of the fourth antibody labeled with ¹²⁵Iodine completes the system, allowing the formation of a bridge between the coated antibodies and the labeled antibody.

After washing, the remaining radioactivity bound to the tubes is directly related to the concentration of TG in the calibrators or samples.

The careful choice of the four monoclonal antibodies allows high specificity and high sensitivity and avoids excess of specificity which is sometimes reproached to immunometric assays with only two monoclonals.

3. REAGENTS PROVIDED WITH THE KIT

- The reagents are sufficient for 100 determinations.
- Store the kit and reagents at 2-8 °C.
- The expiration date of each reagent is shown on the label.

1. **Radioactive Tracer:** 1 vial containing 27.5 ml ¹²⁵I labelled anti-human thyroglobulin mouse monoclonal antibody in phosphate buffer containing gelatin. Radioactivity : 480 kBq. Preservatives : NaN₃ (< 0.1 %), Neomycin and Aprotinin .
2. **Calibrators: calibrator 0** : 1 vial containing 3 ml. Preservative : NaN₃ (< 0.1 %). **Calibrators 1-7** :7 vials containing 1 ml of human thyroglobulin in phosphate buffer containing proteins and protein stabilizers. Preservative : NaN₃ (< 0.1 %). Concentrations: 0 - 0.75 - 1.5 - 5 - 15 - 50 - 200 and 600 ng/ml. **Calibrator at 0.75 ng/ml may be used only with the sensitive method.**
3. **Control:** one vial containing 1 ml of human thyroglobulin in phosphate buffer containing proteins and protein stabilizers. **The exact value is stated on the Quality Control data sheet.** Preservative : NaN₃ (< 0.1 %).
4. **Coated Tubes:** 100 tubes coated with three anti-human thyroglobulin mouse monoclonal antibodies. Unused tubes must be stored at 2-8 °C , protected from moisture.
5. **Washing Solution (50 x concentrated):** 1 vial of 20 ml Tris-HCl buffer with detergent and preservative NaN₃ (< 0.1%). Bring to 1000 ml with distilled water. The diluted washing solution is stable for 2 months at 2-8 °C.

6. **Recovery** : one vial containing 5 ml of human thyroglobulin in phosphate buffer containing proteins and protein stabilizers. The concentration of thyroglobulin is 200 ng/ml. Preservatives : NaN₃<0.1 %.
7. **Diluent** : one vial containing 25 ml of phosphate buffer solution with BSA and preservative NaN₃ (< 0.1 %).

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Plastic test tubes
- Test tube racks.
- Adjustable, automatic micropipettes with disposable tips.
- Vortex mixer.
- Graduated cylinder.
- Aspiration pump or automated washing device.
- Scintillation gamma counter.
- Distilled water.
- Orbital shaker adjustable at 150 rpm.

5. WARNINGS AND PRECAUTIONS

In order to obtain reproducible results, the following rules must be observed :

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.
- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Avoid any contamination among samples; to this purpose disposable tips should be used for each sample and reagent.

In order to avoid personal and environmental contamination, the following precautions must be observed :

- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All material of human origin used for the preparation of this kit has been tested and found negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious; therefore, the assay waste must be decontaminated and disposed of, in accordance with established safety procedures. Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121 °C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.
- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
- Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.
- Acquisition, storage, use and disposal of radioactive material (liquid and solid) are subject to regulation and ordination of local authorities.

6. SPECIMEN COLLECTION

Either serum or plasma may be used. Hemolyzed lipemic sera may give aberrant results. Samples may be stored at 2°-8 °C for up to 24 hours. For long term storage, samples should be divided in aliquots and frozen at -20 °C. Avoid repeated freezing and thawing. After thawing and before use, mix samples by inversion.

Although interferences from anti-thyroglobulin autoantibodies have been greatly reduced by a careful choice of the monoclonal antibodies involved in the assay, it is advisable to verify the validity of the thyroglobulin result by performing a recovery test as described below.

7. ASSAY PROCEDURE

- Bring all reagents and samples to warm up at room temperature.
- Mix samples by gentle agitation before use.
- For all calibrators, a duplicated measure is recommended before use.

A. SENSITIVE ASSAY PROCEDURE

1. Prepare tubes to accommodate Calibrators, test Sera/Plasmas, Recoveries and Control in duplicate. Use non sensitized polystyrene tubes for the measurement of the Total activity.
2. Pipette **100 µl** of the Calibrators, Samples, Recoveries and Control into the appropriate coated tubes. Pipette directly to the bottom of the tubes.
3. Add **100 µl** of Diluent to all these tubes, except the tubes for Total activity.
4. **Mix gently the tubes on vortex and incubate for 2 hours** on an orbital shaker set at 150 rpm.
5. At the end of the incubation, aspirate completely the contents of the tubes. Wash once the tubes with **2 ml** washing solution. **Aspirate completely and remove any residual moisture.**
6. Add **250 µl** of radioactive tracer (red colored) to all the tubes.
7. Incubate the tubes for **18-24 hours** at room temperature.
8. Aspirate carefully the incubation mixture from all tubes except those of Total activity.
9. Wash the tubes twice with **2 ml** of washing solution to all tubes except total tubes. **Aspirate completely the contents of the tubes and remove any residual moisture.**
10. Count the radioactivity bound to the tubes for 1 minute in a gamma counter. We suggest to control the background of the instrument before counting the assay. In order to avoid variations in the sensitivity of the system, the background must be reduced to a minimum or adjusted properly.

SCHEME OF THE ASSAY WITH SENSITIVE PROCEDURE

Tubes	Total Activity	Calibrators	Control	Samples	Recovery
Reagent					
Calibrators	-	100 µl	-	-	-
Control	-	-	100 µl	-	-
Samples	-	-	-	100 µl	-
Recovery	-	-	-	-	100 µl
Diluent	-	100 µl	100 µl	100 µl	100 µl
- Mix on vortex and incubate for 2 h at room temperature shaking (150 rpm). - Aspirate and wash 1 x 2 ml					
Tracer	250 µl	250 µl	250 µl	250 µl	250 µl
- Incubate: 18 – 24 h at room temperature - Aspirate and wash: 2 x 2 ml - Count					

B. NON-SENSITIVE ASSAY PROCEDURE

1. Prepare tubes to accommodate Calibrators, test Sera/Plasmas, Recoveries and Control in duplicate. Use non sensitized polystyrene tubes for the measurement of the Total activity.
2. Pipette **50 µl** of the Calibrators, Samples, Recoveries and Control into the appropriate coated tubes. Pipette directly to the bottom of the tubes.
Do not use the calibrator at 0.75 ng/ml.
3. Add **200 µl** of Diluent to all these tubes, except the tubes for Total activity.
4. Mix gently the tubes on vortex and incubate for **2 hours** on an orbital shaker set at 150 rpm.
5. At the end of the incubation, aspirate completely the contents of the tubes. Wash once the tubes with **2 ml** of washing solution. **Aspirate completely and remove any residual moisture.**
6. Add **250 µl** of radioactive tracer (red colored) to all the tubes.
7. Incubate the tubes for **18-24 hours** at room temperature.
8. Aspirate carefully the incubation mixture from all tubes except those of Total activity.
9. Wash the tubes twice with **2 ml** of washing solution to all tubes except total tubes. **Aspirate completely the contents of the tubes and remove any residual moisture.**
10. Count the radioactivity bound to the tubes for 1 minute in a gamma counter. We suggest to control the background of the

instrument before counting the assay. In order to avoid variations in the sensitivity of the system, the background must be reduced to a minimum or adjusted properly.

SCHEME OF THE ASSAY WITH NON-SENSITIVE PROCEDURE

Tubes	Total Activity	Calibrators	Control	Samples	Recovery
Reagent					
Calibrators	-	50 µl	-	-	-
Control	-	-	50 µl	-	-
Samples	-	-	-	50 µl	-
Recovery	-	-	-	-	50 µl
Diluent	-	200 µl	200 µl	200 µl	200 µl
- Mix on vortex and incubate for 2 h at room temperature shaking (150 rpm). - Aspirate and wash 1 x 2 ml					
Tracer	250 µl	250 µl	250 µl	250 µl	250 µl
- Incubate: 18 – 24 h at room temperature - Aspirate and wash: 2 x 2 ml - Count					

C. RECOVERY TEST

1. Preparation of the recovery samples (spiked samples).

Dilute 1/2 the samples with the contents of the recovery vial, for example **125 µl** sample (**75 µl** with the non-sensitive method) + **125 µl** (**75 µl** with the non-sensitive method) recovery solution. The thyroglobulin concentration in the recovery solution is 200 ng/ml. The so prepared samples are the "spiked samples".

2. Assay of the unspiked samples and of the spiked samples.

In the same serie, measure thyroglobulin in **both** the unspiked and spiked samples.

3. Calculation.

The recovery percentage is calculated as follow :

$$\text{Recovery (\%)} = \frac{\text{Value of the spiked sample}}{(\text{Value of the unspiked sample} + 200)/2} \times 100$$

If there is no interference, the recovery will be near 100 %. If the recovery is lower than 80 %, an interference of TGAB may be supposed and should be verified by measuring TGAB in the sample. If the recovery is higher than 120 %, the observed interference is not due to TGAB.

8. CALCULATION OF THE RESULTS

Draw the calibration curve on log/lin graph by plotting the B/T (%) obtained for each calibrator (y-axis) against the relative concentration (x-axis). Calculate the B/T (%) of each sample and read the concentration by interpolating on the calibration curve.

EXAMPLE OF CALCULATION

The values reported below must be considered as an example and may not be used in place of experimental data

Description	Average cpm.	B/T (%)	hTg conc. (ng/ml)
Total Activity (T)	252413	-	-
CAL 0	188	0,1	0
CAL 1	479	0,2	0.75
CAL 2	835	0,3	1.5
CAL 3	2226	0,9	5
CAL 4	6681	2,6	15
CAL 5	21743	8,6	50
CAL 6	71320	28,1	200
CAL 7	171516	67,5	600
CONTROL	10647	4,2	24.3
P1	3975	1,6	9.0
P2	21344	8,4	49.2
P3	74078	29,1	206.8

9. REFERENCE VALUES

It is recommended that each laboratory determines its own reference interval. Values reported below are only indicative.

The normal values for Thyroglobulin have been determined on 150 samples and Thyroglobulin levels have been found **less than 40 ng/ml**.

10. PERFORMANCE OF THE ASSAY

SENSITIVITY

Analytical sensitivity

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value + 2 S.D.). This dose is 0.1 ng/ml.

Fuctionnal sensitivity

The functional assay sensitivity is the lowest value which is measured with a precision of maximum 20% inter-assay variance. For the h-Tg, this value is lower than 0.1 ng/ml.

PRECISION

Precision was evaluated upon intra- and inter-assay variability at different analyte concentrations.

Intra-assay

Serum	Mean	±	S.D.	C.V. (%)	N
1	9.4	(ng/ml)	0.2	2.1	20
2	51.9	±	0.8	1.5	20
3	112.8	±	1.7	1.5	20

Inter-assay

Serum	Mean	±	S.D.	C.V. (%)	N
1	9.2	(ng/ml)	0.2	2.2	10
2	51.6	±	1.6	3.1	10
3	215.5	±	4.6	2.1	10

ACCURACY

The accuracy of the method has been evaluated by recovery and parallelism tests.

Recovery test

Samples were processed in assays which included one to one dilution with the Thyroglobulin Recovery. The percentages of recovery were calculated. This evaluation was made with 100 samples containing no anti-thyroglobulin auto-antibodies (TgAb negative) and 100 samples containing various levels of anti-thyroglobulin auto-antibodies (TgAb positive) ranging from 50 to more than 5,000 IU/ml.

87 % of samples gave a recovery higher than 80 %. Moreover, no correlation was found between the level of anti-thyroglobulin auto-antibodies and the recovery.

Parallelism

Serums with high analyte concentration were tested at different dilutions with the Zero Calibrator.

Dilution	Expected (ng/ml)	Measured (ng/ml)	Recovery (%)
S1 undiluted	-	161	-
1/2	80.5	88.1	109.4
1/4	40.2	44.7	111.2
1/8	20.1	24.5	121.9
1/16	10.1	12.0	118.8
1/32	5.0	6.4	128.0
S2 undiluted	-	241.5	-
1/2	120.8	121.2	100.3
1/4	60.4	64.0	106.0
1/8	30.2	33.3	110.3
1/16	15.1	15.6	103.3
1/32	7.5	8.7	116.0

HOOK EFFECT

Samples spiked with purified human thyroglobulin up to 100,000 ng/ml gave values higher than the last calibrator in both procedures.

11. BIBLIOGRAPHIE – BIBLIOGRAPHY – ΒΙΒΛΙΟΓΡΑΦΙΑ – BIBLIOGRAFIE – BIBLIOGRAFIA - BIBLIOGRAFÍA

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