

TBG
BL-41-CT

Bio-Line s.a.

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RADIOIMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF THYROXINE BINDING GLOBULIN IN HUMAN SERUM

100 Determinations

FOR IN VITRO DIAGNOSTIC USE ONLY

1. CLINICAL APPLICATIONS

Thyroxine-binding globulin (TBG) is a glycoprotein with a molecular weight of 60000.

The T4/TBG ratio is generally considered as an excellent parameter for assessing thyroid function rather than the Free Thyroxine Index (FTI) which depends on less quantitative measurements given by the T3-Uptake tests.

Therefore, a correct assessment of thyroid function requires simultaneous measurements of TBG, T4 and T3.

Thyroid hormone production, drug administration, age and to a lesser extent sex can influence serum TBG concentrations.

2. PRINCIPLE OF THE ASSAY

In this test kit, TBG of the calibrators or of the samples competes with ¹²⁵I labelled TBG for a limited number of binding sites of a specific antiserum. After incubation, tracer bound to the antiserum is precipitated with a second antibody. After centrifugation and decantation, the radioactivity bound to the precipitate is inversely related to the concentration of TBG in the calibrators or the samples.

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 (10.5 ml) containing ¹²⁵I-TBG. Radioactivity content : 170 kBq per vial Preservative : NaN₃ < 0.1 %.

2. Calibrators:

Zero calibrator 1 vial human serum (lyophilised). Reconstitute with 1.0 ml of distilled water. After reconstitution, calibrators can be stored at 2-8 °C for 2 months. Preservative: NaN₃ < 0.1%.

Calibrators : 5 vials of TBG in human serum (lyophilised). Reconstitute with 0.6 ml of distilled water.

After reconstitution, calibrators can be stored at 2-8 °C for 2 months. Preservative: NaN₃ < 0.1%.

For the exact value, refer to the value indicated on the quality control data sheet

Calibrators have been standardised with the International Standard 88/638 (1 µg/ml = 1 IU/ml).

3. Control: 1 vial of TBG in human serum (lyophilised). Reconstitute with 0.6 ml of distilled water.

After reconstitution, control serum can be stored at 2-8 °C for 2 months. Preservative : NaN₃ < 0.1%.

For the exact value, refer to the value indicated on the quality control data sheet.

4. Antiserum : 1 vial containing 10.5 ml of TBG antiserum raised in rabbit. Preservative : NaN₃ < 0.1 %

5. Precipitating solution : 1 vial containing 100 ml of precipitating solution (sheep anti-rabbit gamma globulins and PEG).

Before use, add the same quantity of distilled water. The solution should be thoroughly stirred before and during use. Preservative : NaN₃ < 0.1 %.

4. MATERIAL REQUIRED BUT NOT SUPPLIED.

- Plastic test tubes
- Test tube racks.
- Graduated cylinder.
- Distilled water
- Adjustable, automatic micropipettes with disposable tips.

- Vortex mixer.
- Refrigerated centrifuge (min 2500 x g)
- Scintillation gamma counter.

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; for 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 tested 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

It is recommended to use serum. Highly lipemic or hemolyzed samples must be discarded. Keep samples at 2-8°C for 1 day; for longer periods it is advisable to freeze samples in aliquots at -20°C. Repeated freezing and thawing of samples should be avoided.

If plasma is used, separate reference values should be created.

7. ASSAY PROCEDURE

- Bring reagents and samples to warm up at room temperature.
- Mix samples by gentle agitation before use.
- For all calibrators, a duplicate measure is recommended.
- Add all reagents in the indicated order.

1. Prepare plain tubes for Total Counts, NSB, Calibrators, Control and Samples.

2. Pipette **25 µl** of each Calibrator, Control and Sample into the corresponding tubes.

3. Add **100 µl** of Radioactive Tracer into all tubes.

4. Add **100 µl** of antiserum into all tubes, except the tubes for Total Counts and NSB. Mix gently.

5. Incubate for **1 hour** at room temperature.

6. Add **2 ml** of precipitating solution (previously diluted) into all tubes except the tubes for Total Counts. Mix gently.

7. Incubate for **10 minutes** at room temperature.

8. Centrifuge at 1,500 g at 4°C for **15 minutes**.

9. Decant the supernatant and count radioactivity in the precipitate.

ASSAY SCHEME

Tubes	Total Activity	NSB	Calibrators	Controls	Samples
Reagent					
Calibrator 0	-	25 µl	-	-	-
Calibrators	-	-	25 µl	-	-
Controls	-	-	-	25 µl	-
Samples	-	-	-	-	25 µl
Tracer	100 µl	100 µl	100 µl	100 µl	100 µl
Antiserum	-	-	100 µl	100 µl	100 µl
- Mix gently and incubate: 1 h R.T.					
Precipitating solution	-	2 ml	2 ml	2 ml	2 ml
- Mix gently and incubate for 10 min at R.T.					
- Centrifuge at 1,500 g for 15 min.					
- Aspirate the supernatant or decant thoroughly.					
- Count the radioactivity of tubes for one minute in a gamma counter.					

8. CALCULATION OF THE RESULTS

First calculate for each duplicate the average NSB-corrected counts per minute.

Calculate the binding of each duplicate as a percent of maximum binding (B_0), with the NSB-corrected cpm.

Using Logit-Log graph paper, plot Percent Bound on the Y-axis against Concentration on the X-axis for each of the calibrators. Draw a straight line approximating the path of the calibration points. Concentrations for the unknowns are then estimated from the line by interpolation.

In case of computer-assisted analysis, a special program suitable for radioimmunoassays and adapted to the counter-computer is to be used.

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/B ₀ (%)	TBG (IU/ml)
Total Activity (T)	79017	-	-
NSB	3430	-	-
CAL 0	66124	100	0
CAL 1	56988	85.4	4
CAL 2	39944	58.2	10
CAL 3	30654	43.4	17
CAL 4	18818	24.5	35
CAL 5	13562	16.2	60
CONTROL	30089	42.5	17
P1	36128	52.5	12.2
P2	24612	33.8	23.6
P3	16372	20.6	44.3

9. REFERENCE VALUES

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

	Mean ± S.D. (IU/ml)	N
Men Healthy	16.9 ± 3.9	100
Women Healthy	21.2 ± 4.9	99
Pregnant Women	35.1 ± 6.9	29
Women under estrogens	22.4 ± 6.9	20
Normal range	16.9 ± 6.2	

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 1.1 IU/ml.

PRECISION

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

Intra-assay

Serum	Mean (IU/ml)	±	S.D.	C.V. (%)	N
1	12.1	±	0.6	5.0	20
2	21.9	±	0.8	3.7	20
3	41.3	±	1.7	4.1	20

Inter-assay

Serum	Mean (IU/ml)	±	S.D.	C.V. (%)	N
1	12.6	±	0.4	3.2	9
2	23.2	±	0.6	2.6	9
3	40.6	±	1.8	4.4	9

ACCURACY

Accuracy of the method has been checked by the recovery and parallelism test

Recovery test: Samples, mixed with equal volumes of each calibration point, were tested.

	MEASURED (IU/ml)	EXPECTED (IU/ml)	RECOVERY (%)
S1	14.2	-	-
S1 + CAL 0	7.4	7.1	103.6
S1 + CAL 1	10.1	9.5	106.1
S1 + CAL 2	12.0	12.1	99.4
S1 + CAL 3	14.5	15.6	92.8
S1 + CAL 4	24.7	24.6	100.4
S1 + CAL 5	45.9	39.6	115.9
S2	18.0	-	-
S2 + CAL 0	9.5	9.0	106.0
S2 + CAL 1	11.8	11.3	103.9
S2 + CAL 2	13.6	14.0	97.2
S2 + CAL 3	17.2	17.5	98.6
S2 + CAL 4	26.1	26.5	98.4
S2 + CAL 5	46.7	41.5	112.6

Parallelism test: Samples with high analyte concentrations were tested at different dilutions with the zero calibrator.

DILUTION	MEASURED (IU/ml)	EXPECTED (IU/ml)	RECOVERY (%)
S1 undiluted	30.6	-	-
1/2	17.1	15.3	111.9
1/4	8.8	7.6	115.3
1/8	3.5	3.8	90.7
S2 undiluted	37.4	-	-
1/2	23.1	18.7	123.7
1/4	12.4	9.3	133.1
1/8	5.3	4.7	114.2

This package insert is downloadable at www.Bio-Line.be

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

1. Attwood E. C. The T3/TBG Ratio and the Biochemical Investigation of Thyrotoxicosis. Clin. Biochem., 1979, **12** (3), 88-92.
2. Gershengorn M. C., Larsen P. R., Robbins J. Radioimmunoassay for Serum Thyroxine-Binding Globulin: Results in Normal Subjects and in Patients with Hepatocellular Carcinoma. J. Clin. Endocrinol. Metab. 1976, **42** (5), 907-911.
3. Hesch R. D., Gatz J., McIntosh C. H. S., Janzen J., Hehrmann R. Radioimmunoassay of thyroxine-binding globulin in human plasma. Clin. Chim. Acta, 1976, **70**, 33-42.
4. Mulaisho C., Utiger R. D. Serum thyroxine-binding globulin: determination by competitive ligand-binding assay in thyroid disease and pregnancy. Acta Endocrinol., 1977, **85**, 314-324.