



TG Ab



IMMUNOENZYMOMETRIC ASSAY FOR THE QUANTITATIVE DETERMINATION OF ANTI-THYROGLOBULIN AUTOANTIBODIES IN HUMAN SERUM AND PLASMA

BL-06-E- 96 Assays

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FOR IN VITRO DIAGNOSTIC USE ONLY

CLINICAL APPLICATIONS AND PRINCIPLE OF THE ASSAY

Autoimmune thyroid diseases encompass a wide spectrum of different clinical Symptoms varying from hypo- to hyperthyroidism (Hashimoto's disease and Graves' disease). The link between these extremes is the presence of serum auto-antibodies directed against the microsomal antigen (TMAB) and/or thyroglobulin (TgAb). Thyroid autoimmunity is more frequently registered in women. Antibody prevalence in women increases with age, rising from approximately 10% at the age of 18-24 up to 30% at the age of 55-65 for TgAb and from 15% at the age of 18-24 up to 24% at the age of 55-65 for TMAB. These significant TgAb and TMAB titers can lead to the development of chronic thyroiditis, often resulting in hypothyroidism. Asymptomatic thyroid diseases with transient hyperthyroidism have been frequently noted in post-partum conditions. Subjects with moderate-sized goiters exhibit high TMAB and/or anti-TPO levels. A rise in TgAb, TMAB and/or anti-TPO has recently been proposed as an independent marker for at "at-risk" pregnancies. In cases of primary myxoedema, significant TgAb and TMAB levels indicate the end-stage of autoimmune atrophic chronic thyroiditis. In younger patients, a firm goiter found in combination with high TgAb and TMAB levels is generally a sign of Hashimoto's disease characterized by a progressive decrease in thyroid function leading to hypothyroidism. In Graves' disease, the toxic goiter is associated to chronic thyroiditis, as confirmed by high TgAb and TMAB levels. Monitoring of TgAb TMAB and anti-TPO levels is frequently used for treatment follow-up. TgAb can also be used as a marker for identification of family members exposed to the risk of transmission of autoimmune conditions.

This test is based on an immunoenzymometric assay (IEMA). The solid phase (polystyrene microwells) is coated with human thyroglobulin. During the first incubation, the autoantibodies possibly present in calibrators and samples are bound to the antigen coated on the solid phase. After washing to remove all unbound material, the tracer is added (anti-hlgG conjugated to horseradish peroxidase) which binds to the antibodies captured during the first step, if any. Following this incubation the unbound material is removed by aspiration and washing. The residual enzyme activity found in the wells will thus be directly proportional to TgAb concentration in calibrators and samples and evidenced by incubating the solid phase with a Chromogen solution (Tetramethylbenzidine, TMB) in a Substrate-Buffer. Colorimetric reading will be performed by using a spectrophotometer at 450 and 405 nm.

REAGENTS PROVIDED WITH THE KIT

- Reagents are sufficient for 96 wells.
 - Store the kit at 2-8°C.
 - The expiry date of each reagent is shown on the vial label.
- 1 - **Coated Microtiterplate:** 96 breakable wells coated with human thyroglobulin. Keep unused wells at 2-8°C, protected from moisture, in the provided aluminum bag and carefully sealed.
 - 2 - **Calibrators:** 6 vials (2 ml) of TgAb in prediluted human serum, at the following concentrations: 0, 50, 100, 500, 1000 and 5000 IU/ml. Ready for use.
Preservative: NaN₃ (<0.1 %). The calibrators have been calibrated against the MRC 65/93 International Reference.
 - 3 - **Enzyme Tracer:** 1 vial (20 ml) of anti-hlgG conjugated to horseradish peroxidase (HRPO), in Tris buffer containing BSA and stabilizers.
Preservative: Neomycin. Ready for use.

- 4 - **Control Serum:** 1 vial (2 ml) of prediluted human serum. Ready for use
Preservative: NaN₃ (<0.1%).
- 5 - **Sample Diluent (concentrated):** 1 vial (30 ml) of phosphate buffer with BSA. Preservative: NaN₃ (<0.1 %). Take vial content to 300 ml with distilled water. The solution is stable for 3 months at 2-8°C.
- 6 - **Washing Solution (concentrated):** 1 vial (100 ml) of Phosphate buffer with detergent Preservative: Thimerosal (<0.1%). Take vial content to 1000 ml with distilled water. The diluted washing solution is stable for 1 month at 2-8°C. If undissolved crystals are detected, put them back into solution by placing the vial at 37°C for a few minutes.
- 7 - **Chromogen:** 1 vial (15 ml) of Tetramethylbenzidine in citrate-phosphate buffer and DMSO.
- 8 - **Substrate Buffer:** 1 vial (15 ml) of citrate-phosphate buffer containing H₂O₂.
Note: Before use, make a 1+1 dilution with equal volumes of Chromogen and Substrate Buffer in glassware. Avoid direct light exposure and use within 1 hour from preparation.
- 9 - **Blocking Reagent:** 1 vial (15 ml) of 1N H₂SO₄. Ready for use.

KIT REAGENTS

Reagents	Quantity	Physical state
Wells	96	Ready for use
Calibrator	6 x 2 ml	Ready for use
Tracer	1 x 20 ml	Ready for use
Control Serum	1 x 2 ml	Ready for use
Sample Diluent	1 x 30 ml	Concentrated 10 x
Washing Solution	1 x 100 ml	Concentrated 10 x
Chromogen	1 x 15 ml	Ready for use
Substrate Buffer	1 x 15 ml	Ready for use
Blocking Reagent	1 x 15 ml	Ready for use

MATERIAL REQUIRED BUT NOT SUPPLIED

- Adjustable, automatic micropipettes with disposable tips.
- Graduated cylinder.
- Dry Heater, adjustable at 37°C ± 1°C.
- Aspiration pump or automated well washing device.
- Microtiterplate spectrophotometer capable of measuring absorbances within a 0-3.0 A interval at 450 and 405 nm.
- Millimetric graph paper.
- Distilled H₂O.

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.

- Chromogen and Blocking Reagent should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.

- 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.

SPECIMEN COLLECTION AND PREPARATION

The assay can be performed in serum or plasma samples. 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. Plasma samples may present fibrin filaments which could interfere with the assay; make sure that samples are always perfectly clear before testing. Repeated freezing and thawing of samples should be avoided.

Sample pre-dilution

Before testing, samples must be diluted 1:301 as follows:
10 µl Sample + 3000 µl Diluent.

Calibrators and Control Serum are already prediluted and ready for use.

ASSAY SCHEME*

Sample predilution: 1/301

Wells	Blank	Calibrator (0-5)	Control Serum	Samples
Reagents				
Calibrator (0 - 5)	----	100 µl	----	----
Control Serum	----	----	100 µl	----
Samples	----	----	----	100 µl
- Incubate: 37°C 60'.				
- Aspirate and wash: 4 X 350 µl.				
Tracer	-----	100 µl	100 µl	100 µl
- Incubate: 37°C 60'				
- Aspirate and wash: 4 x 350 µl				
Chrom-Substrate	100 µl	100 µl	100 µl	100 µl
- Incubate: 37°C 15'.				
Blocking Reagent	100 µl	100 µl	100 µl	100 µl
- Read : 450-405 nm and 620 nm as reference.				

ASSAY PROCEDURE*

- Allow reagents and samples to warm up at room temperature.
- Mix samples by inversion before use.

1. Prepare the wells for: Blank, Calibrators, Control Serum and samples.
2. Pipette **100 µl** of each Calibrator, Control Serum and prediluted sample into the corresponding wells. Pipette directly in the bottom of the wells.
3. Incubate for **60 minutes at 37°C**.
4. Wash the wells **4 times** with **350 µl** of diluted washing solution. Aspirate all liquid from the wells.
5. Add **100 µl** of Enzyme Tracer into all wells, except for the Blank well.
6. Incubate for **60 minutes at 37°C**.
7. Wash the wells **4 times** with **350 µl** of diluted washing solution. Aspirate all liquid from the wells.
8. Pipette **100 µl** of Substrate-Chromogen solution (see reagent paragraph) into all wells.
9. Incubate for **15 minutes at 37°C**, avoid direct light exposure.
10. Pipette **100 µl** of Blocking Reagent into all wells.
11. Read the absorbance of the wells with a possibly bichromatic Spectrophotometer at 450 and 405 nm, with reference wavelength at 620nm (setting the instrument at zero with the Blank well). Reading must be completed within 20 minutes from the end of the assay.

* Please refer to the instrument instruction sheet if using.

CALCULATION OF RESULTS

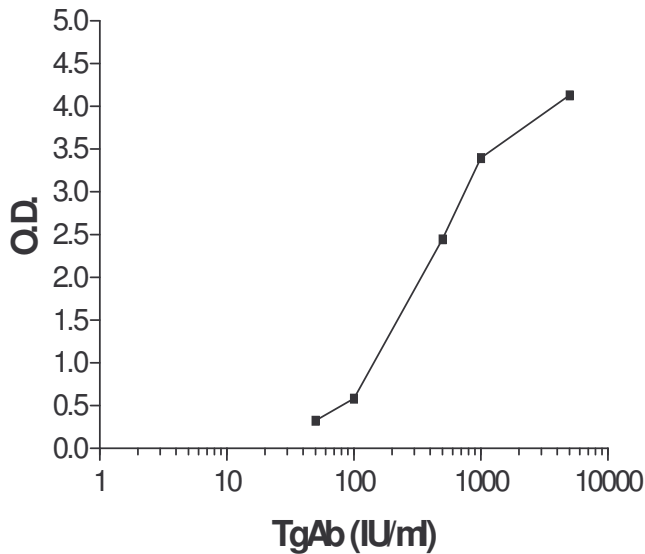
In order to obtain a better sensitivity, the present method employs spectrophotometric reading at two wavelengths (450 and 405 nm). For samples with TgAb concentrations ranging from 0 to 500 IU/ml, read at 450nm; for samples with TgAb levels higher than 500 IU/ml, read at 405 nm. Draw a calibration curve on millimetric graph paper, by plotting the calibration concentration (x-axis) against the absorbance obtained for each standard (y-axis). Corresponding TgAb concentrations in IU/ml are obtained by interpolating the absorbances of each sample on the calibration curve.

EXAMPLE OF CALCULATION

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

Description		O.D.1	O.D.2	O.D.3	O.D. mean	TgAb Conc.
Calibrator 0	IU/ml	0.023	0.010	0.017	0.017	
Calibrator 50	IU/ml	0.331	0.309	0.345	0.328	
Calibrator 100	IU/ml	0.591	0.547	0.619	0.586	
Calibrator 500	IU/ml	2.340	2.459	2.540	2.446	
Calibrator 1000	IU/ml	3.387	3.465	3.336	3.396	
Calibrator 5000	IU/ml	4.221	4.122	4.056	4.133	
Crtl	IU/ml	1.493	1.634	1.589	1.572	281

TYPICAL CALIBRATION CURVE



NORMAL VALUES

The normal values determined on 101 samples, are only indicative since they may be affected by various agents (climate, geographic area, diet, etc.). We recommend that each laboratory establishes its own normal range.

Lower than 90 IU/ml	negative for anti-Thyroglobulin antibodies.
Borderline 90-130 IU/ml	
Higher than 130 IU/ml	positive for anti-Thyroglobulin antibodies.

PERFORMANCES OF THE ASSAY

SPECIFICITY

No cross-reactions have been observed with antibodies against thyroid microsomal antigen, or with other antibodies present in autoimmune diseases.

SENSITIVITY

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Standard (mean value + 2 S.D.). This dose is 2,3 IU/ml.

PRECISION

Precision was evaluated upon intra- and inter-assay variability, in 3 sera at different TgAb concentrations.

Intra-assay

Serum	Mean	± (IU/ml)	S.D.	C.V. (%)	Replicates no.
a	143.4	±	16,9	11,8	20
b	530,8	±	25,3	4,8	20
c	832,1	±	94,8	11,4	20

Inter-assay

Serum	Mean	± (IU/ml)	S.D.	C.V. (%)	Assays no.
a	158.9	±	22.3	14.0	9
b	469.1	±	66.0	14.1	9
c	790.1	±	77.4	9.8	9

ACCURACY

Accuracy of the method has been checked by the recovery and parallelism tests :

Recovery Test

One prediluted (1:301) sample, mixed with equal volumes of each calibration point, were tested.

Samples	Expected (IU/ml)	Measured (IU/ml)	Recovery (%)
S1	---	46	---
S1+50	48	41	85.4
S1+100	73	95	130.1
S1+500	273	275	100.7
S1+1000	523	581	111.1
S1+5000	2509	2261	90.1

Parallelism Test






One serum prediluted (1/301) with high TgAb concentrations were tested at different dilutions with the Zero Calibrator.

Dilution	Expected (IU/ml)	Measured (IU/ml)	Recovery (%)
S1 prediluted	---	652	---
1/2	326	359	110.2
1/4	163	170	104.2
1/8	82	68	83.0

BIBLIOGRAPHY

1. Prentice L.M. et al. Geographical distribution of subclinical autoimmune thyroid disease in Britain : A study using highly sensitive direct assays for autoantibodies to thyroglobulin and thyroid peroxidase. *Acta Endocrinologica* , 1990, 123, 493-8.
2. Jaume J.C. et al. Thyroid peroxidase autoantibody epitopic « fingerprints » in juvenile Hashimoto's thyroiditis : evidence for conservation over time and in families. *Clin. Exp. Immunol.*, 1996, 104, 115-123.
3. Czarnocka B. et al. Immunochemical properties of hTPO. Thyroperoxidase and Thyroid Autoimmunity, Ed. P. Carayon, J. Ruf. 1990, 207, 59-67.
4. Czarnocka B. et al. Majority of thyroid peroxidase autoantibodies in patients with autoimmune thyroid disease are directed to a single TPO domain. *Autoimmunity*, 1996, 23, 145-154.
5. Takamatsu J. et al. Correlation of antithyroglobulin and antithyroid-peroxidase antibody profiles with clinical and ultrasounds characteristics of chronic thyroiditis. *Thyroid*, 1998, 8, 1101-6.
6. Smyth P. et al. Serum thyroid peroxidase autoantibodies, thyroid volume, and outcome in breast carcinoma. *J. Endocrinol. Metab.*, 1998, 83, 2711-6.
7. Gauna A. et al. Immunological aspects of Graves' disease patients in different clinical stages. *J. Endocrinol. Invest.*, 1989, 12, 671-7.
8. Stagnaro-Green A. et al. Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies. *JAMA*, 1990, 264, 1422-5.

Bio-Line aTG-Elisa Kit
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	storage temperature limitation
LOT	batch code
	use by
	consult operating instructions
IVD	<i>in vitro</i> diagnostic device
	manufactured by
REF	catalogue number
SYMBOLS (EDMA recommendations)	
	number of determinations (96)
CAL	Calibrators
CONTROL	Control serum
SORB MTP	Microtiterplate
HRP-CONJ	Enzyme tracer
DIL 10X	Diluent to be diluted ten-fold
CHROM	Chromogen
SUBS	Substrate buffer
BUF WASH 10X	Washing solution to be diluted ten-fold
H₂SO₄ 0.5 M	Blocking reagent