DIAGNOSTIKA GMBH

Instructions for Use

TPO Antibody ELISA

Enzyme Immunoassay for the Quantitative Determination of Autoantibodies to Thyroid-Peroxidase (TPO) in Serum and Plasma

CE

IVD

REF EA619/96

Σ 12 x 8

+2 +8 °C 2 – 8 °C

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Contents

1. Introduction and Principle of the Test

In some diseases of the thyroid, serum autoantibodies against some thyroid antigens are found. The most important antigens are:

- Thyroglobulin (Tg)
- Thyroid peroxidase (TPO), formerly known as microsomal antigen
- TSH-Receptor

Measurement of these autoantibodies is of considerable value in the diagnosis of autoimmune thyroid diseases (Hashimoto-thyroiditis, primary myxedema, hyperthyroidism, asymptomatic autoimmune thyroiditis) and the anti-TPO-ELISA kit provides a convenient method of quantitating TPO autoantibodies in patients' serum or plasma samples.

The assay is based on an easy to use and flexible strip well system employing wells coated with highly purified thyroid peroxidase. Autoantibody binding to the TPO coated wells is detected using Protein A conjugated to alkaline phosphatase as marker and p-nitrophenyl phosphate as substrate. Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared by using known standards (NIBSC66/387).

The same diluted sample can be used for both anti-Tg-ELISA and anti-TPO-ELISA.

2. Precautions

- For in vitro diagnostic use only.
- Do not eat, drink or smoke where immunodiagnostic materials are being handled. Do not pipet by mouth.
- Some reagents contain sodium acid as preservative. Avoid skin contact.
- Wear disposable gloves when handling immunodiagnostic material.
- Some kit components are made with human sera. All sera used were tested for HIV I/II antibodies and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potential biohazardous material.

3. Storage and Stability

Store all reagents at 2 - 8 °C and use before expiry date.

Wash Buffer may be stored at 2 - 8 °C after dilution and used within the shelf life of the kit.

Unused TPO coated strip wells must always be stored at 2 - 8 °C with desiccant in the self-seal bag provided.

4. Contents of the Test

4.1 **MT Strips** **STRIPS** 12 strips
8 wells each, breakable,
coated with thyroid peroxidase

4.2 **Enzyme Conjugate** **CONJ** 1 vial
11 ml, ready for use
Contains Protein A conjugated to alkaline phosphatase.

4.3 **Standards A - E** **CAL A** – **CAL E** 5 vials
1.0 ml each, ready for use

Concentrations (Units are NIBSC 66/387):

Standard	A	B	C	D	E
U/ml	0	5	40	400	5,000

4.4 **Controls 1 and 2** **CON 1 & 2** 2 vials
0.5 ml, concentrates.
Dilute 1:20 with Assay Diluent.
For TPO antibody concentration see QC-Certificate.
For longer storage (more than 4 weeks) freeze Controls at - 20 °C.

4.5 **Wash Buffer** **WASH** 1 bottle
125 ml, concentrate.
Dilute 10 times with distilled water.

4.6 **Assay Diluent** **DIL** 1 bottle
125 ml, ready for use

4.7 **Substrate** **SUB** 1 vial
11 ml pNPP solution, ready for use.

4.8 **Stop Solution** **STOP** 1 vial
10.5 ml, ready for use.
Contains 0.2 M NaOH; 0.25 M EDTA.

Reagents and materials required but not provided:

- Multi Channel Pipette
- Variable Pipets (0 - 100 µl and 0 - 200 µl)
- Microtiter Plate - Reader
- Distilled water

5. Preparation of Reagents and Samples

Allow reagents and required number of MT Strips to reach room temperature. The volumes listed below are for 96 determinations.

Specimens and Controls

Dilute patient sera and Controls 1 and 2 with Assay Diluent 1:20,
e.g. 50 µl + 950 µl. (Do not dilute the ready for use Standards.)

Wash Buffer

Dilute the concentrate 10 times with distilled water (e.g. 100 ml concentrate + 900 ml distilled water).

6. Assay Procedure

6.1 Pipette 50 µl of Standards, diluted Controls and diluted samples into suitable wells of the coated MT Strips. Duplicate determinations are recommended.

6.2 Cover the plate and incubate at room temperature on an ELISA plate shaker (500 shakes per min.) for 15 minutes.

6.3 After the first incubation, invert the plate and briskly shake out the well contents. Fill each well with diluted Wash Buffer dispensed from a laboratory wash bottle, dispenser or pipette. Repeat this procedure 3 times. Remove excess solution by tapping the inverted plate on a paper towel.

6.4 Pipette 100 µl of the Enzyme Conjugate into the wells.

6.5 Cover the plate and incubate at room temperature on an ELISA plate shaker (500 shakes per min.) for 15 minutes.

6.6 Repeat the washing procedure as described in 6.3.

6.7 Pipette 100 µl of Substrate into each well, and incubate for 15 minutes at room temperature in the dark without shaking.

6.8 After this time a yellow-green colour will have developed, and the reaction is stopped by addition of 100 µl of Stop Solution to each well.

6.9 Read the optical density at 405 nm within 30 minutes using a microplate reader blanked preferably against a well containing 100 µl Substrate and 100 µl Stop Solution.

Any test sample reading above the highest standard should be further diluted with Assay Diluent and measured again in the test.

7. Calculation of Results

On a semilogarithmic graph paper the concentration of the Standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each Standard and sample can be related to the optical density of the highest standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis.

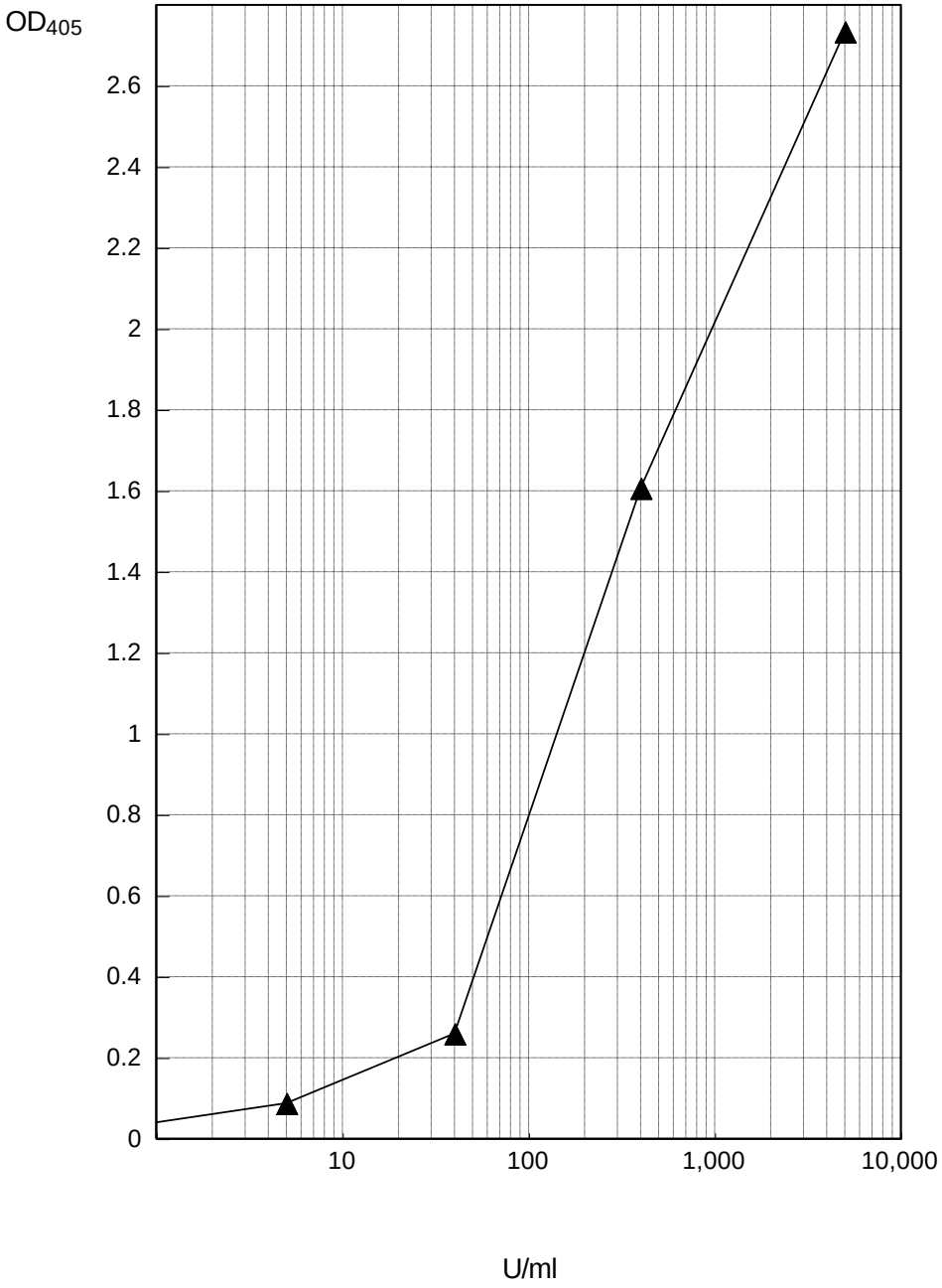
The concentration of the neat samples can be read directly from this standard curve by using their average optical density. The 1:20 predilution of samples and Controls has already been considered in the given concentrations of the Standards.

8. Typical Example

Below is listed a typical example of a standard curve with the anti-TPO-ELISA.

Concentration (U/ml)	Mean OD at 405 nm
0	0.020
5	0.088
40	0.260
400	1.607
5,000	2.734

Typical Standard Curve



9. Assay Characteristics

Assay Cut Off

Negative	< 10 U/ml
Positive	≥ 10 U/ml

Clinical Specificity

Samples from 199 individual healthy blood donors were analysed in the anti-TPO ELISA. 189 (95%) were identified as being negative for TPO autoantibodies.

Clinical Sensitivity

Samples from 66 patients diagnosed with Graves' disease or Hashimoto's were assayed in the anti-TPO ELISA. 50 (76%) were identified as being positive for TPO autoantibodies.

Lower Detection Limit

The zero standard was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at 2 standard deviations was 1.05 U/ml.

Intra-Assay Variation (n=25)

sample	mean U/ml	cv (%)
1	24	6.9
2	78	3.4
3	352	4.8

Inter-Assay Variation (n=20)

sample	mean U/ml	cv (%)
1	12.3	8.1
2	86	5.4
3	194	6.5

Clinical Accuracy

Analysis of sera from patients with autoimmune diseases other than Graves' or Hashimoto's diseases indicated that 14% (n=7) of sera positive for antibodies to GAD, 38% (n=8) of sera positive for antibodies to dsDNA, 36% (n=11) of sera positive for acetylcholine receptor, 75% (n=4) of sera positive for antibodies to 21-OH, and 3% (n=30) of sera positive for Rheumatoid Factor were positive for anti-TPO Ab in the ELISA. No sera positive for antibodies to IA-2 (n=8) or positive for antibodies to AQP4 (n=2) were positive for anti-TPO Ab in the ELISA.

Interference

No interference was observed when samples were spiked with the following materials: haemoglobin up to 500 mg/dl, bilirubin up to 20 mg/dl and intralipid up to 3,000 mg/dl.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for anti-TPO Ab levels.

10. References

- B. Rees Smith (2001);
  Thyroid Autoantibodies
  Scand J Clin Lab Invest 2001 **61** (suppl 235): pp. 45-52.

- P. Burne et al. (2005);
  Point of care assays for autoantibodies to thyroid peroxidase and thyroglobulin"
  Thyroid 2005 **15:** 1005-1010

Pipetting Scheme

Standards (A to E)	50 µl
Controls 1:20 diluted	50 µl
Samples 1:20 diluted	50 µl

↓

15 minutes incubation at room temperature
on an ELISA plate shaker (500 shakes per min.)

3 x washing with Wash Buffer

↓

Conjugate	100 µl

↓

15 minutes incubation at room temperature
on an ELISA plate shaker (500 shakes per min.)

↓

3 x washing with Wash Buffer

↓

Substrate	100 µl

↓

15 minutes incubation at room temperature
in the dark without shaking

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Stop Solution	100 µl

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Reading of absorbance at 405 nm