INSTRUCTION MANUAL

IVD

(July 25, 2014)

Medizym® anti-TPO

- 96 determinations -

REF **3002**

CE

Immunenzymometric assay for the determination of autoantibodies to Thyroid Peroxidase (TPO ab) in human serum

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IFU symbols non-radioactive assays MEDIPAN GMBH

IVD	In vitro diagnostic device	DIL	Sample diluent
REF	Catalogue number	CE	EC Declaration of Conformity
	Expiry date	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Store at		Consult operating instruction
MP	Coated microtiterplate (96 wells)		Biological risk
WASHB	Wash buffer	SUB	Substrate
CAL	Calibrators	CONJ	Conjugate
STOP	Stop solution	CONTROL	Control serum

INTENDED USE

Medizym® anti-TPO is used for the quantitative determination of thyroid peroxidase autoantibodies (TPO ab) in human serum. TPO itself is a membrane-bound enzyme in the thyroid cell. It is essential for the thyroid hormone synthesis and is one of the three thyroid antigens.

One of the characteristics of autoimmune thyroid diseases is the presence of autoantibodies to thyroid antigens.

Consequently, the determination is indicated for the detection of chronic autoimmune thyroiditis and for the differential diagnosis of hypothyroidism including its subclinical or latent type. The anti-TPO determination is also valuable in Graves' disease, particularly if the TSH Receptor Antibody value is negative, but the disease is clinically suspected. Another indication is the exclusion of a co-existing thyroid autoimmune disease in case of euthyroid goiter.

PRINCIPLE of the TEST

Medizym® anti-TPO is an immunenzymometric solid-phase assay for the quantitative determination of autoantibodies to thyroid peroxidase (TPO ab) in serum.

The autoantibodies of the controls, standards, and diluted patient samples react with recombinant thyroid peroxidase insolubilized on the solid phase of microtiter plates. Highly purified recombinant TPO coated on the microtiter plate guarantees the exclusive binding of TPO autoantibodies of the specimen under investigation. Following an incubation period of 60 min, unbound serum components are removed by a washing step.

The bound autoantibodies react specifically with anti-human IgG antibodies conjugated to horse radish peroxidase (HRP). Following an incubation period of 30 min, excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

The horse radish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 15 min, turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific autoantibodies bound. The standard curve is plotted by using the concentrations of the autoantibodies of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of autoantibodies of the specimen is directly read off from the standard curve. Evaluating the test by a semi-quantitative method is also possible.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Do not use lipaemic and hemolytic samples. Plasma is not suitable.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v / v),***
e.g. 10 µl sample + 1 ml sample diluent (G), prior to assay
(calibrators and controls of the kit are ready for use, prediluted accordingly).

TEST COMPONENTS
for 96 DETERMINATIONS

A MP	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with recombinant human thyroid peroxidase		1 each well color coded green
B WASHB	**Concentrated wash buffer** sufficient for 1000 ml solution		100 ml Concentrate capped white
G DIL	**Sample diluent**		100 ml ready for use capped black
D CONJ	**Conjugate** containing polyclonal anti-human-IgG (sheep) coupled with horse radish peroxidase		15 ml ready for use capped red
E SUB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide		15 ml ready for use capped blue
F STOP	**Stop solution** 0.25 M sulfuric acid		15 ml ready for use capped yellow
1 - 4 CAL	**anti-TPO calibrators** (diluted serum) conc.: 50, 300, 1000, 3000 IU/ml	L	1 ml ready for use capped white
CI CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed	L	1 ml ready for use capped green
CII CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed	L	1 ml ready for use capped red

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
  trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

Size and storage

Medizym® anti-TPO has been designed for 96 determinations. This is sufficient for the analysis of 42 unknown samples as well as for calibrators and control sera, assayed in duplicates.
The expiry date of each component is reported on its respective label, that of the complete kit on the box label.
Upon receipt, all components of the Medizym® anti-TPO have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow all of the components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer 10 times (1 + 9) by de-ionized or distilled water. For example, dilute 20 ml of the concentrate with 180 ml of distilled water. The washing solution prepared is stable at 2 - 8 °C up to 30 days.

Avoid exposure of the substrate to light.

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (G) 1 + 100 (v/v), e.g. 10 µl serum + 1 ml sample diluent (G)**.
- **Duplicates are recommended.**
- **Avoid any time shift during pipetting of reagents and samples**

1. Bring all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators (1 - 4) or **100 µl** of calibrator 1 (semi-quantitative)
   100 µl control sera (CI, CII)
   100 µl diluted patient samples into the respective wells.
3. Incubate **60 min** at room temperature.
4. Decant, then wash each well **three** times using **300 µl** washing solution (prepared from B).
5. Add **100 µl** of conjugate (D) to each well.
6. Incubate **30 min** at room temperature.
7. Decant, then wash each well **three** times using **300 µl** washing solution (prepared from B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min *in the dark*** at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the optical density at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

Medizym® anti-TPO allows both the quantitative (4 calibrators) and semi-quantitative (calibrator 1 for cut-off determination) evaluation of the results.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 on the ordinate, y-axis, versus their respective anti-TPO-concentrations on the abscissa, x-axis.

The anti-TPO concentrations of the controls and the unknown samples are directly read off in IU/ml against the respective OD values.

Medizym® anti-TPO may also be used with Computer Assisted Analysis using software able to curves with log / lin processing.

Using the recommended dilution of 1 + 100 (v+ v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results are interpreted by calculating the binding index (BI) using calibrator 1 (50 IU/ml) as cut-off control. The BI is the ratio of the OD-value of the sample to the OD value of the calibrator 1.

$BI = OD_{sample} / OD_{calibrator\ 1}$

TYPICAL EXAMPLE

Do not use for evaluation!

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Wells	OD (a)	OD (b)	OD (mean)	IU/ml
Calibrator **1**	0.320	0.318	0.322	**50**
Calibrator **2**	1.041	1.061	1.051	**300**
Calibrator **3**	1.592	1.628	1.610	**1000**
Calibrator **4**	2.202	2.259	2.231	**3000**
Controls CI, CII	---	---	---	---
Patient 1	0.541	0.573	0.557	**115**

STANDARD CURVE

Typical example

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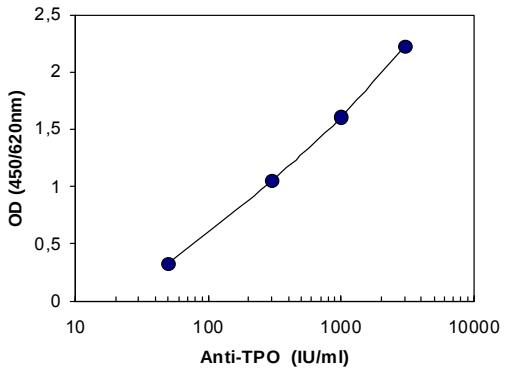

REFERENCE VALUES

Medizym® anti-TPO	IU / ml	BI
Positive	≥ 50	≥ 1
negative	< 50	< 1

More than 90 % of the cases of autoimmune thyroiditis are anti-TPO positive. In active Graves' disease, the proportion of anti-TPO positive results is above 70 %.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-TPO levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned data only provide a guide to values which might be expected.

Even low positive values of anti-TPO indicate that autoimmune processes occur. However, this does not inevitably mean that any thyroidal dysfunction already exists or is clinically manifest.

Subacute thyroiditis De Quervain normally results as anti-TPO negative.

CHARACTERISTIC ASSAY DATA

Calibration

Medizym® anti-TPO is calibrated against NIBSC-reference serum 66/387, (NIBSC: Nat. Inst. for Biol. Standards and Control, Hampstead, London, U.K.).

Parallelism of standards and serum samples

Defined dilutions of the reference material with anti-TPO free human serum are found congruent to calculation with Medizym® anti-TPO. Human sera with high anti-TPO levels are also recognized correctly within the working range of Medizym® anti-TPO.

Sensitivity (Limit of Detection)

The analytical sensitivity was determined as 5 IU/ml.

Specificity

The high quality of the insolubilized recombinant TPO ensures the exclusive reaction of TPO autoantibodies as well as the absence of any detectable cross-reactions with autoantibodies to thyroglobulin (cf. Medizym® anti-Tg) or to TSH receptor (cf. TSH-REZAK®).

Functional Assay Sensitivity / Limit of Quantitation

The most appropriate and statistically reasonable definition of the lower detection limit of any assay is at present the so-called **functional assay sensitivity**.

This functional assay sensitivity generally represents that concentration which corresponds to the 20 % (between assay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range.

Upon correct and thorough performance of Medizym® anti-TPO, the limit of quantitation is found at approx. 9 IU/ml.

Anti-TPO values below this defined level do not meet the statistical criteria for reliability according to GLP (good laboratory practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

Anti-TPO concentrations above approx. 9 IU/ml, however, are consequently assessed as valid.

Precision

Intraassay (n = 20)		**Interassay(n = 5x10)**	
Mean IU/ml	CV %	Mean IU/ml	CV %
1570	6,5	1753	6,8
304	5,9	295	2,5
88	5,5	80	7,2

LIMITATIONS of the METHOD

Healthy individuals should be tested negative by the Medizym® anti-TPO. However, TPO autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Medizym® anti-TPO

INCUBATION SCHEME

Dilute patient sera* **10 µl serum + 1 ml sample diluent (G)**

Step	Activity	Material	CAL 1 – 4 or CAL 1 alone	CI, CII	Patients 1, 2, … prediluted
1	Bring all reagents to room temperature before use.				
2	Pipette	Samples	100 µl	100 µl	100 µl
3	Incubate	Plate	60 minutes (room temperature)		
4	Wash	Washing solution	3 x 300 µl	3 x 300 µl	3 x 300 µl
5	Pipette	Conjugate (D)	100 µl	100 µl	100 µl
6	Incubate	Plate	30 minutes (room temperature)		
7	Wash	Washing solution	3 x 300 µl	3 x 300 µl	3 x 300 µl
8	Pipette	Substrate (E)	100 µl	100 µl	100 µl
9	Incubate	Plate	15 minutes (room temperature) in the dark		
10	Pipette	Stop solution (F)	100 µl	100 µl	100 µl
11	Measure OD	Plate	at 450 nm versus 620 (690) nm		

*** This dilution is also used for Medizym® anti-Tg**

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (≤ 1% v/v) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.