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IVD



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2°C-8°C



Σ=96 tests

REF

#1422-2

TPO IgG ELISA TEST SYSTEM

An Enzyme-Linked Immunosorbent Assay (ELISA) for IgG antibodies to Thyroid Peroxidase (TPO)

Catalog No. 1422-2For *In Vitro* Diagnostic Use

INTENDED USE

The Diagnostic Automation, Inc. TPO IgG ELISA test system is intended for the qualitative and semi-quantitative detection of IgG-class antibody to thyroid peroxidase (TPO) in human serum. The test system is intended to be used as an aid in the diagnosis of thyroid diseases. This test is for *in vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Thyroid antibodies are a characteristic finding in patients with Hashimoto's and Graves' diseases (1). The presence of thyroid antibodies in the sera of 80% of patients with these two diseases led to the recommendation that some type of thyroid antibody testing be a feature of the work-up of any patient with a goiter (1). Although thyroid antibodies are predominantly associated with Hashimoto's or Graves' diseases, they may be found in the sera of patients with other diseases such as myxedema, granulomatous thyroiditis, nontoxic nodular goiter, and thyroid carcinoma (1). Thyroid antibodies are also found in most cases of lymphocytic thyroiditis in children (2), and rarely in patients with pernicious anemia and Sjögren's Syndrome (3-4).

PRINCIPLE OF THE ELISA ASSAY

The Diagnostic Automation, Inc. TPO ELISA test system is designed to detect IgG class antibodies to TPO (thyroid peroxidase) antibodies in human sera. Wells of plastic microwell strips are sensitized by passive absorption with TPO antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microwells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

KIT COMPONENTS

Reactive Reagents:

1. Twelve, 1 x 8-well strips coated with purified human thyroid peroxidase ($\geq 98\%$ pure). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Horseradish peroxidase conjugated goat anti-human IgG. Ready to use. One 15 mL vial. Preservative added.
3. Human positive serum control. One 0.35mL vial. Preservative added.
4. Human negative serum control. One 0.35mL vial. Preservative added.
5. Calibrator A. One 0.35 mL vial. Preservative added.
6. Calibrator B. One 0.35 mL vial. Preservative added.
7. Calibrator C. One 0.35 mL vial. Preservative added.
8. Calibrator D. One 0.35 mL vial. Preservative added.
9. Sample diluent. One 30mL bottle containing Tween-20 bovine serum albumin, and phosphate-buffered-saline, pH 7.2 \pm 0.2. Ready to use. (Green solution with a green cap; Product No. 005N).
10. TMB: One 15mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO < 15% (w).
11. Stop solution: One 15mL bottle (red cap) containing 1M H₂SO₄, 0.7M HCl. Ready to use.
12. Wash buffer(10X): One 100mL bottle containing phosphate-buffered-saline and Tween-20. 10X concentrate. Dilute 1 part concentrate and 9 parts deionized or distilled water. NOTE: 1X solution will have a pH of 7.2 \pm 0.2. Preservative added.

Note: Kit also contains:

1. Data labels: One label is adhered to the inside lid of the kit box and a second label is inside the kit box.
2. Package insert providing instructions for use.

Materials required but not provided:

- ELISA microwell reader capable of reading at a wavelength of 450nm
- Pipettes capable of accurately delivering 10 to 200 μ L.
- Multichannel pipette capable of accurately delivering (50-200 μ L).
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps
- Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

PRECAUTIONS**Safety Precautions:**

1. The Diagnostic Automation, Inc. Anti-TPO IgG ELISA kit is for *in vitro* diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
2. The TPO plate wells do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
3. The controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HbsAg, and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories":1993, pp 12-16, 3rd edition; and OSHA's Standard for Bloodborne Pathogens (5).
4. Controls, Sample Diluent, Conjugate, and Wash Buffer contain preservative (thimerosal, 0.04% (w/v)) which may be toxic if ingested.
5. The sample diluent contains sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
6. Stop solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
7. TMB Substrate is HARMFUL. Irritating to eyes, respiratory system and skin.
8. Wash Buffer (10X) is an IRRITANT. Irritating to eyes, respiratory system and skin.

Procedure Precautions:

1. For *in vitro* diagnostic use.
2. Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
3. Dilution or adulteration of these reagents may generate erroneous results.
4. Reagents from other sources or manufacturers should not be used.
5. TMB solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB substrate solution with conjugate or other oxidants will cause the solution to change color prematurely. Do not use substrate solution if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of substrate solution to be used.
6. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
7. Avoid microbial contamination of reagents. Incorrect results may occur.
8. Cross contamination of reagents and/or samples could cause erroneous results
9. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
10. Strict adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20-25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
11. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration), before adding conjugate or substrate. Do not allow the wells to dry out between incubations.
12. Avoid splashing or generation of aerosols.

13. Do not expose reagents to strong light during storage or incubation.
14. Allowing the multiwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
15. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
16. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
17. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
18. Do not allow the conjugate to come in contact with containers or instruments which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
19. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C,
2. Coated microwell strips: Store at 2-8°C. Extra strips should be immediately resealed with desiccant and returned to storage at 2-8°C. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Peroxidase conjugated goat anti-human antibody: Store at 2-8°C. DO NOT FREEZE.
4. Human controls: Store at 2-8°C.
5. TMB substrate solution: Store at 2-8°C.
6. Wash buffer: Store at 2-25°C. Diluted wash buffer is stable for 30 days at 2-8°C, or 7 days at 2-25°C.
7. Sample diluent: Store at 2-8°C.
8. Stop Solution: Store at 2-25°C

SPECIMEN COLLECTION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (6, 7). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

PROCEDURE

Preparation of Reagents:

1. Wash Buffer: Dilute the 100mL of 10X concentrate with 900mL of distilled or deionized water. Mix thoroughly to dissolve any crystals that may be present.
2. Sample diluent, substrate solution, conjugate, and stop solution are ready to use.

TEST PROCEDURE

Set-up of the Assay:

Step1 Reagents preparation: Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative controls (e.g. B1, C1, D1) two Positive controls (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Example of controls/samples dispensing scheme											
	1	2	3	4	5	6	7	12	
A	Blank	S3									
B	Neg.	...									
C	Neg	...									
D	Neg.										
E	Pos.										
F	Pos.										
G	S1										
H	S2										

- Step3** Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient Serum. The sample diluent will undergo a color change confirming that the specimen has been combined with the diluent.
- Step4** To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
- Step5** **Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.**
- Step6** **Incubate the plate at room temperature (20-25°C) for 25±5 minutes.**
- Step7** **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5times** with Wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remaining liquids. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.
- Step8** Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
- Step9** Incubate the plate at room temperature (20-25°C) for 25 ± 5 min.
- Step10** Wash the microwells by following the procedure as described in step 7.
- Step11** Add 100µL of TMB to each well, including the reagent blank well, at the same rate and in the same order as the specimens were added.
- Step12** **Incubate** the plate at room temperature for 10 to 15 minutes.
- Step13** Stop the reaction by adding 50µL to Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop solution, tap the plate several times to ensure that the samples are thoroughly mixed.
- Step14** Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

QUALITY CONTROL

- Each time the assay is run, the positive control, negative control, calibrator A, B, C, and D must be included. Additionally, a reagent blank must be included.
- The specifications for the positive and negative controls are as follows:

Positive Control	Must be > 30 IU/mL
Negative Control	Must be < 15 IU/mL

 - The OD of the negative control divided by the OD of the positive control should be ≤ 0.9 .
 - If the control values are not within the above ranges, the test should be considered invalid and the test should be repeated.
- The positive control is intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

1. Calibrator

Based upon testing of normal sera, disease state sera, and the World Health Organization (WHO) international standard (66/387), a maximum normal IU/mL value has been determined by Diagnostic Automation Inc., and correlated to the calibrators. The calibrators will allow you to determine the unit value for each of the test samples evaluated. The unit values are determined for each lot of kit produced, and are printed on the data label included with each kit.

2. Conversion of Optical Density to IU/mL

Optical densities of the specimens are determined from the standard curve generated from the calibrators. A standard curve should be generated using the paired data points for each of the four calibrators (OD on the Y axis and corresponding IU/mL value on the X axis). Using the best fit, point to point curve, determine the IU/mL value for each of the specimens tested by extrapolation.

3. Quality Control

Refer to the specification sheet included with each kit. This sheet describes the lot specific specifications for each of the calibrators. If any of the calibrators are out of range, the results are considered invalid, and the patient results may not be reported.

INTERPRETATION OF RESULTS:

Using normal healthy individuals, disease-state specimens, and the WHO standard, Diagnostic Automation, Inc., has established the following guidelines for interpretation of patient results:

< 25 IU/mL	Negative
25-30 IU/mL	Equivocal*
> 30 IU/mL	Positive

* Specimens which are repeatedly equivocal should be tested using an alternate serological method.

LIMITATION OF THE ASSAY

1. A diagnosis should not be made solely on the basis of the ELISA result. Test results for anti-thyroglobulin should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Reproducible results with an ELISA system require careful pipetting, strict adherence to incubation periods and temperatures requirements, as well as thorough washing of the test wells and thorough mixing of all solutions.
3. Hemolytic, icteric, or lipemic samples may interfere with this ELISA. Use of these types of specimens should be avoided.

EXPECTED RESULTS

The clinical investigation included 80 random normal donor specimens. With respect to this group, four (5%) were positive and 76 (95%) were negative.

PERFORMANCE CHARACTERISTICS

Comparative Study:

An in-house comparative study was performed to determine the equivalence of the Diagnostic Automation, Inc. TPO IgG ELISA test system to another commercially available TPO IgG ELISA test system. Performance was evaluated using 248 specimens and the results have been summarized in Table 1 below.

Table 1. Summary of the comparative investigation, DAI ELISA test system versus a commercially available ELISA test system.

		DAI TPO IgG ELISA Test System			
		Positive	Equivocal*	Negative	Totals
Commercial TPO IgG ELISA Test System	Positive	104	12	7	123
	Negative	7	1	117	125
	Totals	111	13	124	248

- Equivocal specimens were excluded from calculations below.

Relative* Sensitivity = $104/111 = 93.7\%$ - 95% Confidence Interval of 89.1 to 98.2%
Relative Specificity = $117/124 = 94.4\%$ - 95% Confidence Interval of 90.3 to 98.4%
Relative Agreement = $221/235 = 94.0\%$ - 95% Confidence Interval of 91.0 to 97.1%

* Please be advised that the term 'relative' refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

Reproducibility

A study was conducted in-house to determine reproducibility. Briefly, six specimens were tested; two negative, two strong positive, and two positive specimens that were near the assay cut off. Each specimen was tested in eight replicate wells on each day, for a total of three days. The resulting data was used to determine both intra and inter assay reproducibility. A summary of the study appears in Table 2 below.

Table 2. Results of three day reproducibility study.

	Intra-Assay						Inter-Assay	
	Day One		Day Two		Day Three		Three Days Combined	
	Mean(IU/mL)	% CV	Mean(IU/mL)	% CV	Mean(IU/mL)	% CV	Mean(IU/mL)	% CV
Sample 1	77	3.1	55	3.9	79	5.9	69	15
Sample 2	112	5.8	88	16.4	109	3.0	103	12.6
Sample 3	32	9.2	37	3.8	35	11.8	34	10.7
Sample 4	34	4.9	40	1.2	35	12.2	36	12.1
Sample 5	13	2.4	12	1.9	9	10.4	11	18.4
Sample 6	9	6.0	6	1.3	5	17.6	6	30.6

Cross Reactivity.

To evaluate the system for potential cross reactivity to other autoantibodies, seventeen specimens which were positive for antibodies to nuclear antigens (ANA) on HEp-2 cells were tested. Of the specimens tested, none were positive on the TPO IgG ELISA. This study indicates that the potential for interference due to cross reactive autoantibodies is unlikely.

Correlation to the World Health Standard (NIBSC 66/387)

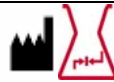
The World Health Standard (NIBSC 66/387) was tested on the DAI assay to determine the correlation of the result obtained to the expected result. The data from this study is presented in Table 3 below.

Table 3. Correlation to the World Health Standard; (NIBSC 66/387)

Dilution of the Standard	IU/mL as Tested	OD (450 nm)	Result: (IU/mL)
neat	1000	> 3.000	278
1:2	500	> 3.000	275
1:4	250	2.710	243
1:8	125	1.740	144
1:16	62	0.890	61
1:32	32	0.457	30
1:64	16	0.202	14
1:128	8	0.112	8

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