ImmunoDOT™

CE

THYROID AUTOIMMUNITY TEST

IVD **For *In Vitro* Diagnostic Use**

INTENDED USE

The ImmunoDOT Thyroid Autoimmunity Panel is an enzyme immunoassay (EIA) test for screening and detection of autoantibodies against human thyroglobulin and human thyroid peroxidase (microsome) in serum and heparinized whole blood and is used as an aid in the diagnosis of thyroid disorders. This product is intended for use in physician offices and clinical laboratories.

SUMMARY AND EXPLANATION

Autoimmune thyroid gland disorders are characterized by detection of anti-thyroid antibodies, primarily against thyroglobulin and/or microsomal thyroid antigens. Recently it has been shown that thyroid peroxidase (TPO) is the protein responsible for microsomal antigenicity[1]. In addition to chronic thyroiditis, thyroid autoantibodies may be found in other thyroid disorders. These autoantibodies may also occur in apparently normal subjects.

Thyroid microsomal (TPO) autoantibodies occur in sera of most autoimmune thyroid disease patients and predict raised serum TSH levels in random populations[2]. The presence of autoantibody does not imply active tissue destruction[2,3]. Microsomal (TPO) antibody level correlates with the degree of lymphoid infiltration of the thyroid gland[2,4].

Thyroid autoantibodies are detected by a variety of immunoassays. Two common methods are indirect hemagglutination (IHA) and indirect fluorescent antibody (IFA) techniques. ImmunoDOT Thyroid Autoimmunity Test uses highly purified human thyroglobulin devoid of microsomal antigen and recombinant human thyroid peroxidase (microsomal antigen) which does not contain contaminating thyroglobulin and/or mitochondria found in other microsome antigen preparations. These purified antigens are used in a dipstick format enzyme immunoassay technique.

ASSAY PRINCIPLE

The ImmunoDOT Test utilizes an EIA dot technique for the detection of antibodies. The antigens are dispensed as discrete dots onto a solid membrane. After adding specimen to a reaction vessel, an assay strip is inserted, allowing patient antibodies reactive with the test antigen to bind to the strip's solid support membrane. In the second stage, the reaction is enhanced by removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated anti-human antibodies are allowed to react with bound patient antibodies. Finally, the strip is transferred to enzyme substrate reagent which reacts with bound alkaline phosphatase to produce an easily seen, distinct dot.

REAGENTS

Assay Strip. positive human control, two levels of recombinant human thyroid peroxidase (TPO) protein (microsome), two levels of highly purified human thyroglobulin, and negative control.

Diluent (#1). buffered diluent (pH 6.2-7.6),protein stabilizers with <0.1% NaN_3.

Enhancer (#2). sodium chloride with <0.1% NaN_3.

Conjugate(#3). alkaline phosphatase conjugated goat anti-human antibodies in buffered diluent (pH 6.2-8.5) with <0.1% NaN_3.

Developer(#4). 5-bromo-4-chloro-3-indolyl phosphate and p-nitro blue tetrazolium chloride in buffered diluent (pH 9.0-11.0), 0.8% N, N-Dimethylformamide, and <0.1% NaN_3.

Warnings and Precautions

For In-Vitro Diagnostic Use. ImmunoDOT Thyroid Autoimmunity Panel reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoDOT Assay System reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Analytic quality deionized or distilled water must be used as Clarifier. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since these may result in poor assay performance.

Some assay components contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Warning - Potential Biohazardous Material. Human sera used in the preparation of controls were tested and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease[5].

Storage

Store reagents and assay strips at 2-8°C. Reagents must be at room temperature (15-30°C) before use. Reagents must be used within one hour of placement in the heated workstation. Avoid contamination of reagents which may produce invalid results.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT Thyroid Autoimmunity Test can be performed on either serum or heparinized whole blood. The test requires approximately 10 µL of serum or 20 µL of whole blood.

Serum and heparinized whole blood are collected according to standard practices. Finger stick samples are stable at ambient temperature for one day. Serum and heparinized whole blood may be stored at 2-8°C for up to five days. Serum may be frozen below -20°C for extended periods. Freezing whole blood samples is not advised.

PROCEDURE

Materials Provided

- ImmunoDOT Thyroid Assay Strips
- Diluent (#1)
- Enhancer (#2)
- Conjugate (#3)
- Developer (#4)
- Reaction Vessels
- Package Insert

Materials Required But Not Provided

- GenBio Workstation
- Specimen collection apparatus (e.g., finger sticking device, venipuncture equipment)
- Timer
- Analytic quality distilled or deionized water to be used as Clarifier
- Pipets
- Absorbent toweling to blot dry assay strip
- Positive control serum

Set-Up

1. Turn on Workstation and adjust to proper temperature if necessary. Refer to Workstation Instructions.
2. Remove 4 Reaction Vessels (per test) from the product box and insert into appropriate slots in Workstation. For the large Workstation, add water up to the fill line of the Clarifier vessel provided. For the small Workstation, use an appropriate container and sufficient water to cover all reactive windows of the assay strip.
3. Place 2 mL Diluent (#1) in Reaction Vessel #1; 2 mL Enhancer (#2) in Reaction Vessel #2; 2 mL Conjugate (#3) in Reaction Vessel #3; and 2 mL Developer (#4) in Reaction Vessel #4.
4. Wait ten minutes before beginning "Assay Procedure". During this time, specimen(s) may be added (step #5), Assay Strips labeled (step #6), and inserted into the Strip Holder (step #7).
5. Add patient specimen (approximately 10 µL serum or 20 µL of whole blood) to Reaction Vessel #1.
6. Appropriately label the Assay Strips.
7. If the large Workstation is used, insert the label end of the Assay Strip into the Strip Holder, one per groove, taking care not to touch the assay windows.

Assay Procedure

1. Prewet Assay Strip by immersing in Clarifier for 30 - 60 seconds.
2. Using several (5 - 10) quick up and down motions with the Assay Strip, mix reagent and specimen thoroughly in Reaction Vessel #1. Let stand for 5 minutes.
3. Remove Assay Strip from Reaction Vessel and swish in the Clarifier. Use a swift back and forth motion for 5 - 10 seconds allowing for optimal washing of the Assay Strip's membrane windows.
4. Place Assay Strip into Reaction Vessel #2. Mix thoroughly with several (5 - 10) quick up and down motions. Let stand for 5 minutes.
5. Remove Assay Strip from Reaction Vessel #2 and swish in Clarifier as described (step #3).
6. Place Assay Strip into Reaction Vessel #3. Mix thoroughly with several (5 - 10) quick up and down motions. Let stand for 15 minutes.
7. Remove Assay Strip from Reaction Vessel #3 and swish in Clarifier as described (step #3). DO NOT remove the Assay Strip from the Clarifier.
8. Allow the Assay Strip to stand in the Clarifier for 5 minutes.
9. Remove Assay Strip from Clarifier and place into Reaction Vessel #4. Mix thoroughly with several (5 - 10) quick up and down motions. Let stand for 5 minutes.
10. Remove Assay Strip from Reaction Vessel #4 and swish in Clarifier as described (step #3).
11. Blot and allow Assay Strip to dry. It is imperative that tests of borderline specimens be interpreted after the Assay Strip has been allowed to dry.

Reading the Assay Strip

Positive	A dot with an **EASILY SEEN**, distinct border is visible in the center of the window. The outer perimeter of the window must be white to pale gray.
Negative	If no dot is seen or a dot is difficult to see, interpret it as negative.

Quality Control

The top and bottom membrane windows of the Assay Strip are reagent controls. The top window is a positive reagent control and must be positive for further interpretation. The bottom window is the reagent negative control and must be negative for further interpretation. Reagent controls assure that reagents are active and that the test has been performed properly. If either reagent control is invalid, the test must be repeated. The intensity of the positive control dot must not be used as a calibrator. Positive reactions in the antigen windows of the strip may be either darker or lighter than the positive control depending on the antibody titer.

GenBio quality assures that each kit lot performs as described. In addition, a positive control serum (Product No. 3912), moderately positive for anti-thyroglobulin and anti-thyroid peroxidase, is available separately. The performance of each kit lot may be confirmed upon receipt by running a determination using the positive control serum and obtaining a positive result.

The assay's reagent temperature is between 42-48°C. Due to heat transfer loss, the Workstation temperature is set higher. The appropriate Workstation temperature setting is listed in the Workstation's package insert. (Contact Technical Services for additional guidance if an alternate heat source is used.)

INTERPRETATION

There are two reaction levels for each analyte (thyroglobulin and TPO). Interpret each analyte reaction separately and according to the following criteria:

No Positive Dots	Nonreactive for autoantibody
One Positive Dot	Weakly reactive: Likely to be positive, but follow-up test(s) recommended.
Two Positive Dots	Reactive: Specific autoimmune antibody present.

The antigen levels on the assay strip are set so that nonreactive and reactive outcomes are highly reliable while weakly reactive samples are interpreted as "borderline" results.

LIMITATIONS

Epidemiologic factors, clinical findings, and other laboratory results should be considered in addition to autoantibody laboratory results for diagnosis of the patient.

Thyroid autoantibodies may be present in non-thyroid disorders such as Sjögren's syndrome, pernicious anemia, Addison's disease, myasthenia gravis and diabetes mellitus[6,7,8] and in apparently healthy subjects[2].

PERFORMANCE CHARACTERISTICS

ImmunoDOT Thyroid Autoimmunity Test was tested with presumptive normal samples and microsome and thyroglobulin positive (IHA) samples. The results in normal samples (n=125) predict assay specificity in a random, normal population and are shown in Table 1. These data predict the TPO assay to be 91% specific and the thyroglobulin to be 96% specific. These data are consistent with previous reports[2,6,7,8].

Table 1: Normal Samples

Assay Type	NonReactive	Weak	Reactive
TPO	91.2% (114/125)	6.4% (8/125)	2.4% (3/125)
Thyroglobulin	96% (120/125)	4.0% (5/125)	0% (0/125)

Sixty-six (66) indirect hemagglutination (IHA) positive samples were evaluated in the ImmunoDOT Thyroid Autoimmunity Test for anti-TPO (microsome) to assess test sensitivity compared to the classic test method. These results are shown in Table 2.

Table 2: Thyroid Peroxidase (microsome)

IHA Titer	NonReactive	Weak	Reactive
100	0	4	1
400	0	6	12
1600	0	0	28
6400	0	0	9
25,600	0	0	6

Assuming that an IHA titer greater than or equal to 1:1600 is clinically relevant, the ImmunoDOT Thyroid Autoimmunity Test for anti-TPO (microsome) is 100% (43/43) sensitive when compared to IHA. Unlike the IHA method, ImmunoDOT identified 13 of the 23 borderline (1:100-400) IHA results as clearly reactive samples.

Twenty-one thyroglobulin (Tg) IHA positive samples were tested in the ImmunoDOT Thyroid Autoimmunity Test for anti-thyroglobulin. These results are shown in Table 3.

Table 3: Thyroglobulin

IHA Titer	NonReactive	Weak	Reactive
80	1	0	2
160	1	1	0
320	0	2	2
640	0	0	4
>1280	0	0	8

Assuming that an IHA titer greater than or equal to 1:160 is clinically relevant, the ImmunoDOT Thyroid Autoimmunity Test for anti-thyroglobulin is 94% (17/18) sensitive when compared to IHA. In addition, ImmunoDOT identified two out of three borderline (1:80) IHA positives as clearly reactive samples.

Bibliography

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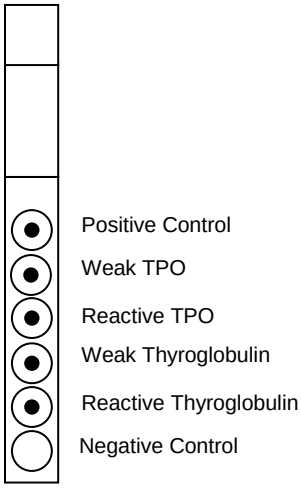

QUICK REFERENCE PROCEDURE

ImmunoDOT Thyroid

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the Clarifier Vessel.
- Place 2 mL Diluent (1) in Vessel #1; 2 mL Enhancer (2) in Vessel #2; 2 mL Conjugate (3) in Vessel #3; and 2 mL Developer (4) in Vessel #4.
- Wait 10 minutes

Procedure

- Add 10 µL serum to Vessel #1.
- **Prewet** assay strip in Clarifier for 30 - 60 seconds.
- Place strip in Vessel #1, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #2, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #3, mix, **let stand 15 min.**
- Remove strip, place in Clarifier, **let stand 5 min**.
- Place strip in Vessel #4, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish, blot, dry, and read.

To place an order for ImmunoDOT products, contact your local distributor or call GenBio directly for the distributor nearest you and for additional product information.

For assistance, please call toll-free 800-288-4368.

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