

# Thyroid Autoimmunity Test (thyroglobulin and recombinant TPO antigens)

The **ImmunoDOT** Thyroid Autoimmunity Test 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. 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<sup>1</sup>. In addition to chronic thyroiditis, thyroid autoantibodies may be found in other thyroid disorders. These autoantibodies may also occur in apparently normal subjects.

## Expected Results

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

Thyroid autoantibodies are detected by a variety of immunoassays. Two common methods are indirect hemagglutination (IHA) and indirect fluorescent antibody (IFA) techniques. **ImmunoDOT** Thyroid Auto-immunity 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.

## Principle

The **ImmunoDOT** test utilizes an enzyme-linked immunoassay (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.

## Performance Characteristics

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. 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. 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.

**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. The results show the TPO assay to be 91% specific and the thyroglobulin to be 96% specific. These data are consistent with previous reports<sup>2,6,7,8</sup>.

## Procedural Summary

1. Put appropriate reagents in Reaction Vessels # 1-4 in workstation.
2. Add 10  $\mu$ L patient serum to Reaction Vessel #1.
3. Prewet Assay Strip in distilled water. Place in Reaction Vessel #1, mix, and incubate 5 minutes.
4. Wash in distilled water.
5. Place Assay Strip into Reaction Vessel #2, mix, and incubate 5 minutes.
6. Wash in distilled water.
7. Place Assay Strip into Reaction Vessel #3, mix, and incubate 15 minutes.
8. Wash and soak in distilled water for 5 minutes.
9. Place into Reaction Vessel #4, mix, and incubate for 5 minutes.
10. Wash in distilled water.
11. Blot and allow Assay Strip to dry. Read results.

## Ordering Information

Product Description	Size (Type)	GenBio Product No.
ImmunoDOT Thyroid Autoimmunity Panel	25 test kit	5825
	100 test kit	5889
Thyroid Positive Control Serum	10 test	3912
Workstation 4 place (120V)* Workstation 12 place (120V)*	4 patient	4011
	12 patient	4090

\*International voltages available

## References

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4. Doniach D. "Humoral and genetic aspects of thyroid autoimmunity" Clin Endocrinol Metab 4:267 (1975)
5. Centers for Disease Control/National Institutes of Health Manual Biosafety in Microbiological and Biomedical Laboratories (1984)
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