DRG

DRG® Anti-Thyroglobulin ELISA (EIA-1613)

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IVD

INTENDED USE

The DRG Anti-Thyroglobulin ELISA test kit is intended for In Vitro determination of the levels of thyroglobulin autoantibodies in human serum.

INTRODUCTION

Thyroglobulin (Tg), the principal storage protein normally present in the thyroid follicles, is composed of a 19S glycoprotein, thyroxine (T4), triiodothyronine (T3) and their precursors. Tg may spill into the circulation as a result of thyroid disease such as Hashimoto's thyroiditis, and cancer, and may deceive the immune system into producing anti-Tg autoantibodies (Tg Ab's). Anti-Tg autoantibodies belong mainly to the immunoglobulin G (IgG) class. The concentrations of circulating Tg Ab's vary over a wide range depending on the causative disease. Therefore, quantitation of circulating Tg Ab's is important in the diagnosis as well as the follow-up of these thyroid diseases. Circulating Tg Ab's occur, in varying amounts, in patients suffering from such diseases as autoimmune thyroiditis due to Hashimoto's Disease, Graves' Disease, endemic goiter, subacute thyroiditis, and thyroid carcinoma. The differential diagnosis is further refined by measuring Thyroid Peroxidase autoantibodies, and Thyrotropin Receptor Autoantibodies (TR Ab's) and Thyroglobulin in serum.
Passive hemagglutination (PHA) is one of the most commonly used tests for measure Tg Ab's. It suffers from (a) subjective interpretation of results, (b) diminished sensitivity, (c) errors caused by large numbers of dilutions, and (d) interference by intrinsic serum factors. The PHA test may miss subclinical thyroiditis, where concentrations of circulating Tg Ab's may be lower than the detection limit of the test. The Anti-Tg ELISA, by virtue of its greater analytical sensitivity, can help identify those patients with subclinical thyroiditis.

PRINCIPLE OF ANTI-THYROGLOBULIN ELISA FOR THYROGLOBULIN AUTOANTIBODIES

The Anti-Thyroglobulin (ELISA) procedure is a quantitative procedure based on the specific binding of purified thyroglobulin immobilized on the microwells of the microtiter plate to the endogenous autoantibodies to thyroglobulin in the patient serum. First, the human autoantibodies to thyroglobulin in the patient serum bind to the purified thyroglobulin on the microwells. After a washing step to the purified thyroglobulin on the microwells. after a washing step to eliminate the excess serum proteins, an enzyme labeled Protein A is added to bind to the antigen-antibody complex in the microwells by virtue of its ability to bind to IgG antibodies. Excess enzyme is eliminated by a second wash step. Subsequent color is developed by the addition of a substrate. The intensity of the color is directly proportional to the amount of auto antibodies to thyroglobulin present in the patient sample. The color developed is measured and quantitated by reading against a Dose Response Curve (DRC).

WARNING AND PRECAUTIONS

All reagents provided with the kit are for *in vitro* diagnostic use only.

WARNING

Potential Biohazardous Material

The matrix of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV ½ and anti-HCV when tested with FDA licensed reagents. Because there is no test method

that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious.

Sodium Azide
The reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. To prevent the possible formation of explosive metal azides, dispose of the reagents by flushing with large amounts of water through the plumbing system.
Stopping Solution
Stopping Solution consists of 1N H_2SO_4. This is a strong acid and should be handled with caution. It can cause burns and should be handled with gloves while wearing eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

REAGENTS AND MATERIALS

Materials Supplied

DRG's Anti-Tg ELISA contains sufficient reagents to run 96 assay wells.

1. Thyroglobulin Coated Microwells.....1 x 96 tests
2. Anti-Human IgG HRP Conjugate.....1 x 10 ml
3. Serum Diluent (10X Conc.)…….…….....1 x 20 ml
4. Washer Buffer (Conc.).....1 x 20 ml
5. Anti-Tg Calibrators
   (0, 30, 100, 300, 1000, & 5000 U/ml).....6 x 1 ml
6. Substrate Solution A (TMB)…….....1 x 8 ml
7. Substrate Solution B (H_2O_2)…….....1 x 8 ml
8. Stopping Solution (1N Sulfuric Acid)…….....1 x 6 ml

All reagents (except #3,4) are supplied ready to use form.
Calibrators are referenced to the International Standard 65/93 for Anti-thyroglobulin Human Serum established by the Medical Research Council.

Storage and Stability

Store all reagents at 2-8°C. The stability of the reagents is stated on the vial label. Do not interchange reagents between kit lots.

Materials Required But Not Supplied

1. Microwell plate reader capable of reading at 450nm.
2. Calibrated semi automatic micropipets to deliver 25, 50, 100 µl and 1.0 ml.
3. Disposable borosilicate glass tubes 13 x 100 mm.
4. Automated microplate washer or squeeze bottle to wash plates.

SPECIMEN COLLECTION AND STABILITY

Collect whole blood by venipuncture using evacuated tubes. Allow the clot to form at room temperature, centrifuge immediately, and separate the serum from the clot. Identify properly and store at 2-8°C if the test is to be run the same day, otherwise, store the serum frozen until assay time.

Reagent Preparation and Storage

1. Serum Diluent

Pour the contents of the **Serum Diluent Concentrate** vial into a 200 ml flask. Wash the contents of the vial into the flask with distilled water and Q.S to the 200 ml mark with distilled water. Label as **Working Serum Diluent.** Store at 2-8°C until kit expiration.

2. Wash Buffer Solution

NOTE: The wash buffer concentrate when stored at 2-8°C may have some crystals at the bottom of the vial. Add the entire contents of the concentrate to 980 ml of distilled water in a 1 liter container (rinse out all crystals). Mix thoroughly. Store at 2-8°C until kit expiration.

3. Substrate Solution

Mix Substrate Solution A and B in the ratio of 1:1 in a 13 x 100 glass tube. For 6 microwell strips (48 wells) mix 3 ml of Substrate Solution A with 3 ml of Substrate Solution B. Mix well before use. Use within 1 hour after preparation.

4. Patient Sample Dilution

Accurately pipet 50 µl of each solution (see number 1 above). Mix thoroughly by inversion or vortexing. the diluted patient sample is stable for 48 hours at 2-8°C. Keep the patient serum sample frozen for any future reference.

NOTE: If a sample reads more than 5000 U/ml, it can be further diluted 1:10 and 1:100 using the original patient dilution already prepared as described above. The value obtained should be multiplied by the dilution factor.

TEST PROTOCOL

Preparation and Precautions

1. Bring all reagents and patient samples to room temperature before use.
2. It is recommended that all calibrators, patient samples, and controls be assayed in duplicate.
3. External controls should be run with the test to verify the precision and accuracy of the method.
4. All incubation times, temperatures, and reagent volumes are critical to achieve accurate results.
5. Return all unused reagents to the refrigerator immediately after use.
6. The supplied reagents in each kit must be used together. No interchanging of reagents from different kit lots should be attempted.
7. Do not use reagents that are past the expiration date.

ASSAY PROCEDURE

1. Assemble the microwell strips needed in the plate holder.
2. Pipet 100 µl of **Calibrators, Controls, and diluted Patient Samples** into the microwells.
3. Cover the microwells and incubate at **37°C for 60 minutes.**
4. Using an automatic or manual plate washing technique aspirate and wash the contents of the wells three times with **wash buffer solution**. If an automatic plate washer is used, wash the wells three times with **300 µl of washer buffer solution** according to the instrument manufacturer's instructions. If manual washing is used, fill a squeeze bottle with **wash buffer solution**. Discard the contents of all the wells into a sink by quick decantation. Carefully fill the wells one by one with the wash buffer solution (avoid air bubbles in the wells during washing), discard the wash solution, blot dry with a paper towel, and then repeat the procedure two more times. Make sure that the plate is blotted dry each time between washes.
5. Add **100 µl of Protein A-HRP Conjugate** to all the wells.
6. Cover the plate and incubate at **37°C for 30 minutes**.
7. Wash the microwells as in **step #4** above.
8. Add **100 µl of working substrate solution** to all the wells.
9. Cover the plate and incubate at **37°C for 15 minutes**.
10. Stop the reaction by adding 50 µl of **Stopping Solution** to all the wells. the blue color in the wells will turn yellow. The yellow color in the wells is

stable for 30 minutes, and the wells should be read within this time.

11. Set the Microplate reader at 450nm and read the plate.

Calculation of Results

1. Record the optical density readings (O.D.) for all wells.
2. Calculate the mean O.D. value of the duplicates.
3. Plot the Dose Response Curve using the mean O.D. (on the Y axis against the Calibrator concentration units on the X axis) using semi-log graph paper.
4. Extrapolate the patient results from the Dose Response Curve.

TABLE 1
Sample Calculations

I.D.	Sample O.D.	Mean O.D.	Value Units/ml	Conc. U/ml
Calib. 0	0.109	0.109	0.109	
Calib. 30 U/ml	0.367	0.352	0.360	
Calib. 100 U/ml	0.653	0.721	0.687	
Calib. 300 U/ml	1.235	1.283	1.259	
Calib. 1000 U/ml	1.634	1.657	1.646	
Calib. 5000 U/ml	2.401	2.274	2.338	
Control #1	0.476	0.513	0.495	57
Control #2	1.036	1.088	1.062	203
Patient #1	0.311	0.276	0.294	20
Patient #2	1.892	1.930	1.911	2008

QUALITY CONTROL

Control sera used in the assay must fall within their specified ranges.
The laboratory should prepare confirmed negative and positive serum pools to be run each time to validate the assay. Alternatively, a commercially available set of controls can be run each time for validation of the assay.

INTERPRETATION OF RESULTS

The results are expressed in International Units. DRG's Anti-Tg ELISA Dose Responce Curve (DRC) is calibrated against international standard IS 65/93 established by the Medical Research Council.

Values greater than 100 Units/ml are considered as positive for Thyroglobulin Autoantibody. However, the clinical meaning of the test results should be interpeted by considering other factors, such as abnormalities in the patient's health and the result of other tests.

The results of this test cannot be interchanged with the PHA test because of the capability of ELISA to detect very low levels of autoantibodies to thyroglobulin.

Expected Values

Disease	Tg Ab	MC Ab
Hashimoto's Thyroiditis	4+	4+
Graves' Disease	1+	3+
Opthalamic Graves' Disease	1+	2+
Toxic Nodular Goiter	±	+
Eutheroid Goiter	±	+
Thyroid Carcinoma	±	±
Active thyroid autoimmune disease	2+	+
Thyrotoxicosis	+	+

LIMITATIONS AND IMPORTANT NOTES

1. As in any *in vitro* diagnostic test, the thyroglobulin autoantibody assay data obtained with this procedure should be used as an aid to other medically established procedures and be interpreted in conjunction with other clinical data available to the physician.
2. Adherence to the protocol and accuracy in pipetting the reagents are essential to obtain good and valid results.
3. Samples which are lipemic or hemolyzed should not be used in the assay. Samples which are contaminated microbiologically likewise should not be used in the assay.
4. Do not mix different lots of any component within an individual assay. Do not use components beyond the expiration date shown on the outside label.
5. Failure to obtain the appropriate anti-thyroglobulin values for the controls may indicate imprecise manipulations, improper sample handling or reagent deterioration.

PERFORMANCE CHARACTERISTICS

Cross-reactivity:

Interference from ANA, DNA and microsomal antibodies, and rheumatoid factors was found to be negligible.

Precision:

The reliability of DRG's Anti-Thyroglobulin ELISA procedure was assessed by examining its reproducibility on samples selected to represent a spectrum of thyroglobulin autoantibody levels.

Within run (Intra-Assay)- Statistics were calculated for each of three samples from the result of 24 pairs of wells in a single run.

Sample	Mean	S.D.	C.V.	n
1	171	0.21	12.53	24
2	679	0.51	7.47	24
2	1093	1.33	12.17	24

Inter-Assay - Statistics were calculated for each of the three samples from the result of pairs of wells in 10 different runs.

Sample	Mean	S.D.	C.V.	n
1	245	0.29	11.78	10
2	720	0.86	11.93	10
2	1185	0.99	8.35	10

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