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Anti-ThyRoglobulin (Anti-Tg)

The Quantitative Determination of Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma. Measurements of Tg autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

REF

Catalog No.E2-022

IVD

FOR INVITRO DIAGNOSTIC USE ONLY

SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroglobulin have been shown to be characteristically present from patients with thyroidits and primary thyrotoxicosis1 ° This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction Passive Hemaglutnation (PHA) methods have been employed in the past for measurements of antibodies to Tg. PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation This procedure, with the enhanced sensitivity of 514 permits the detectability of subclinical levels of antibodies to Tg. In addition! the results am quantitated by spectmphotometer, which eliminates subjective interpretation.

Immunospec's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in this method! serum reference, diluted patient specimen! or control is first added to a microplate well biotinylated thyroglobulin (Tg) is added! and then the reactants are mixed. Reaction results between the autoantibodies to Tg and the biotinylated Tg to form an immune complex! which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human lgG conjugate is then added to permit quantitation of reaction through interacting with human lgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimens enzyme activity can be correlated with autoimmune antibody level.

REAGENTS

Materials Provided:

A. Calibrators -
Six (6) vials of refemnces for anti-Tg. Refer to vials label for exact concentrations. Store at 2-6°C.

B. Thyroglobulin Biotin Reagent
One (1) vial of biotinylated thyroglobulin stabilized in a buffered matrix. A preservative has been added. Store at 2-5°C.

C. Anti-Tg Enzyme Conjugate
Store at 2-6°C.

D. Microplat Coated wells
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-5°C.

E. Serum Diluent-
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-5°C.

F. Wash Solution
One (1) vial containing a surfactant in buffered saline preservative has been added. Store at 2-5°C.

G. solution A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-5°C.

H. Solution B
One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-5°C.

I. Stop Solution-
One (1) bottle of stop solution containing a strong acid HCI). Store at 2-8°C.

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date
Note 2: Opened magenta are stable for sixty (60) days when stored at 2-5°C.
Note 3: Above reagents am for a single 96-well microplate.

Required But Not Provided:

I. Pipette capable of delivering l0µl & 50µl volumes with precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.l00 ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplats washers or a squeeze bottles (optional).
4. Microplate Readermith450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate walls.
6. Plastic wrap or microplats cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
5. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

PRECAUTIONS

For In Vitro Diagnostics use only
Nor for internal or external Use hi Humans or Animals
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable & transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988Publicaton No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-6°C for a maximum period of five (5) days if the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of-20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, of 0.100 ml of the specimen is required.

REAGENT PREPARATION

I. Serum Diluent

Dilute the serum diluent to 200m1 in a suitable container with distilled or deionized water Stow at 2-5°C.
2 Wash Buffer
Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.
3. Working Substrate Solution
Pour the contents of the am bar vial labeled Solution A into the clear vial labeled Solution 5! Place the yellow cap on the clear vial for easy identification Mix and label accordingly. Store at2-8°C.
Note: Do not use the working substrate if it looks blue.
4. Patient Sample Dilution (1/100)
Dispense 0.010ml (10µl) of each patient specimen into 1 ml of serum diluent Cover and vortex or mix thoroughly by inversion. Store at 2-5°C for up to forty-eight (48) hours

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).
1. Format the microplates' wells for each calibrator, control and patent sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at2-8°C.
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µ1) of the Tg Biotin Reagent
4. Swirl the microplate gentlyfor20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplats by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300 µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacture's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the mash and repeat two (2) additional times.
8. Add 0.100 ml (100µI) of x-Tg Enzyme Conjugate to all wells. Always add reagents in the same order to minimize reaction time differences between wells.
Do NOT SHARE OHE PLATE AFOER ENZYME ADDIOON
9. Swirl the microplate gently, cover and incubate for thirty (30) minutes at room temperature.
10. Repeat steps (6 & 7) as explained above
I1. Add 0.100 ml (100 µl) of working substrate to all wells (See Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.
Do NOT SNAKE THE PLATE AFTER SUBSTRATE AODITON
12. Incubate at room temperature for fifteen (15) minutes.
13. Add 0.051ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.
Note: For re-assaying specimens with concentration greater than 2000 lU/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-Tg in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2 Plot the absorbance for each duplicate serum reference versus the corresponding anti-Tg activity in lU/ml on linear graph paper
3. Draw the best-fit curve through the plotted points.
4 To determine the level of anti-Tg activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in lU/me from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.387)) intersects the dose response curve at (790 lU/me anti-Tg concentration (See Figure 1).

Sample I.D.	Well Number	RLU(A)	Mean RUL (B)	Value(U/ml)
Cal A	A1	0.022	0.025	0
	B1	0.028		
Cal B	C1	0.135	0.133	50
	D1	0.131		
Cal C	E1	0.280	0.270	125
	F1	0.261		
Cal D	G1	0.962	0.949	500
	H1	0.936		
Cal E	A2	1.709	1.703	1000
	B2	1.698		
Cal F	C2	2.730	2.698	2500
	D2	2.667		
Cont 1	E2	1.390	1.387	790
	F2	1.383		

*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay

Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 'F' should be≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed Quality control charts should be maintained to follow the performance of the supplied reagents Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limas. In addition! maximum absorbance should be consistent with past experience Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of 4 reagents Fresh reagents should be used to determine the reason for the variations.

LIMITATIONS OF PROCEDURE

A. Assay Performance
1. It is important that the time of reaction in each well is held constant for reproducible results Pipeting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, is recommended to repeat the dose response curve.
2 Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be performed in the same sequence to eliminate any time deviation during reaction.
3. Plate readers measure vertically. Do not touch the bottom of the wells
4 Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results
5. Very high concentration of anti-Tg in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
6. Samples, which are contaminated microbiologically, should not be used.

B. Interpretation

1. If computer controlled data reduction is used to interpret the results of the test, It is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. The presence of autoantibodies to Tg is confirmed when the serum level exceeds 125 lU/ml. The clinical significance of the result, coupled with anti-thyroid peroxidase activity, should be used in evaluating the thyroid condition However; clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests
3. The cost benefits should be considered in the use of thyroglobulin antibodies testing when performed in concert with anti- thyroid peroxidase (TPO). The widespread practice of performing both tests has been questioned'.

EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the Anti-Tg test system. The number (n) mean (x) and standard deviation (ὸ) are given in

TABLE I
Expected Values for the Anti-Tg ELISA Test System (In IU/ml)

Number	100
Mean	74.3
Standard deviation	25.2
Upper 95% (+2σ) level	124.7

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

A. Precision
The within and between assay precisions of the anti-Tg ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value x, standard deviation (a) and coefficient of variation (C.V) for each of these control sera are presented in Table 2 and Table

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	65.5	3.3	5.0%
Pool 2	20	385.5	15.5	4.0%
Pool 3	20	1554.4	55.4	3.6%

TABLE 3*
Between Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	66.8	3.6	5.3%
Pool 2	10	374.2	18.5	4.9%
Pool 3	10	1625.5	65.2	4.0%

*As measured in ten experiments in duplicate.

B. Accuracy
The anti-Tg ELISA test system was compared with a reference anti-Tg ELISA. Biological specimens from normals and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimen was 101. The least square regression equation and the correlation coefficient were computed for the anti-Tg method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	415.6	y = 9.79 + 0.969 (x)	0.995
Reference	419.2		

Only slight amounts of bias between the anti-Tg method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity
The anti-Tg has a sensitivity of 5 lU/ml.

D. Specificity
Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system.

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