Anti – Thyroglobulin IgG

Enzyme Inmunoassay Test Kit

SUMMARY OF ASSAY PROCEDURE

Step	(20 - 25°C Room Temp)	Volume	Incubation time
1	Sample dilution 1:40 4µl / 200 µl		
2	Diluted samples,calibrator & controls	100µl	30 minutes
3	Whasing buffer (3 times)	300µl	
4	Enzyme conjugate	100µl	30 minutes
5	Whasing buffer (3 times)	350 µl	
6	TMB Chromogenic substrate	100µl	15 minutes
7	Stop solution	100µl	
8	Reading OD 450 nm		

Name and intended use

The Anti – Thyroglobulin ELISA is intended for the detection and semi-quantitation of antibodies to thyroglubilin in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of thyroid autoimmune disease.

Summary and explanation of the test

Three major autoantibodies are important in autoimmune thyroid diseases, including anti-Thyroglobulin (Anti – Tg), anti –Thyroid Peroxidase (Anti – TPO) and autoantibody to Thyroid stimulating hormone receptor (Anti – TSHR) (1). Thyroglobulin is a water soluble glycoprotein consisting of two polypeptide homodimers of mw 330,000 that is involved in the storage and synthesis of thyroid hormones.

Anti-Tg are found about 80% of patients with Hashimoto's thyroiditis, 60% with Graves' disease, 30% thyroid Carcinoma, pernicious anemia or sjogren's syndrome, 3% to 18% of apparently normal individuals may also have Anti-Tg (2).

Anti- Tg is targeted against thyroglobulin within thyroid gland follicles. Several methods are available for measuring Anti- Tg including quantitative passive hemagglutination, immunofluoresence and ELISA.

The ELISA with the high sensitivity and specificity permits the measurement of subclinical levels of antibodies to Tg and eliminates subjective interpretation (3, 4, 5)

PRINCIPLE OF THE TEST

Purified Thyroglobulin antigens are coated on the surface of microwells. Diluted patient serum, calibrators and controls are added to wells, and the Thyroglobulin specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, anti-human IgG bind to the antibody-antigen complex. Excess enzyme conjugates are washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibodies in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell strips: Thyroglobulin antigen coated wells. (12 x 8 wells)
2. Sample Diluent : 1 vial (22 ml)
3. Calibrator: Factor value (f) stated on label. 1 vial (150 µl)
4. Negative Control: Range stated on label. 1 vial (150 µl)
5. Positive Control: Range stated on label. 1 vial (150 µl)
6. Washing Concentrate 20x: 1 bottle (50 ml)
7. Enzyme Conjugate: Red color solution. 1 vial (12 ml)
8. TMB Chromogenic Substrate: Amber bottle. 1 vial (12 ml)
9. Stop Solution. 1 vial (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. It is recommend to use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as 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 at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.
Prepare washing buffer by adding distilled or deionized water to 20x wash concentrate to a final volume of 1 liter.
2.Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5µl of the samples, negative control, positive control and calibrator to 200µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop solution to stop reaction.

Make sure there are no air bubbles in each well before reading

9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. To obtain Cut-off OD value: Multiply the OD of Calibrator by Factor (f) printed on label of Calibrator.
2. Calculate the IgG Index of each determination by dividing the OD values of each sample by obtained OD value of Cut-off.

For example:

If Factor (f) value on label = 0.4

This factor (f) is a variable.
It is specific for a lot manufactured and printed on label of Calibrator.

Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition IgG Index = 1)

Patient sample O.D. = 0.580
IgG Index = 0.580 / 0.44 = 1.32 (Positive result)

Patient sample O.D. = 0.320
IgG Index = 0.320 / 0.44 = 0.73 (Negative result)

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.

PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET041

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2. If the O.D. value of the Calibrator is lower than 1.0, the test is not valid and must be repeated.
3. The Anti-Thyroglobulin IgG Index for Negative and Positive Control should be in the range stated on the labels.
4. Each laboratory should assay internal controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION
Negative: IgG Index of 0.90 or less are seronegative for IgG antibody.

Equivocal: IgG Index of 0.91 - 0.99 are equivocal. Sample should be retested.

Positive:IgG Index of 1.00 or greater.

EXPECTED VALUES AND PREVALENCE
Eighty-two (82) specimens from random asymptomatic blood donors were tested with the Anti-Tg IgG ELISA kit. 1 was found to be positive (1.2% positivity) and eighty-one (81) were found to be negative (98.8%).

LIMITATIONS OF THE PROCEDURE
As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS
Specificity and Accuracy:
Specificity and relative accuracy were evaluated using a commercial available ELISA kit and the Anti-Thyroglobulin IgG ELISA kit on eighty-two specimens. The results were summarized in the following table:

		Reference ELISA		
		N	P	Total
Anti-Thyroglobulin IgG ELISA	N	79 (D)	2(B)	81
	P	0 (C)	1 (A)	1
	Total	79	3	82

Specificity = D / (C+D) = 79 / 79 = 100 %

Accuracy (Overall agreement) = (A+D) / (A+B+C+D) = 80 / 82 = 97.5 %

REFERENCES

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