Thyroid Peroxidase Antibody (Anti-TPO) Test System Product Code: 1175-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroid Peroxidase (TPO) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroid peroxidase have been characteristically present from patients with Hashimoto Thyroiditis (95%), diaphragmatic diaphragmatic apnea (90%),

In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction. This has led to clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Measures of antibodies to TPO have in the past been done by Passive Hemaglutination (PHA). PHA tests do not have the sensitivity of chemiluminescence immunoassay and are limited by subjective reaction of biotin and streptavidin in the test. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This becomes a valuable tool in the diagnosis of thyroid dysfunction. 2 This has lead to clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Materials Provided:

E. Serum Diluent Concentrate – 20ml/vial – No Icon

One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

I. Product Insert.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette(s) capable of delivering 0.025ml (25µl) & 0.100ml (100µl), 0.350ml (350µl) and 1.0ml (1000µl) volumes with a precision of better than ±1.5%

2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl).

3. Microplate washer (optional) or squeeze bottle to wash plates.

4. Microplate luminometer.

5. Test tubes for dilution of patient samples and reagents A and B.

6. Absorbent Paper for blotting the microplate wells.

7. Plastic wrap or microplate cover for incubation steps.

8. Vacuum aspirator (optional) for wash steps.


5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All Monobind products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2, and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially infectious and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the current edition of the “Guidelines for Laboratory Practices in Transfusion Medicine.”

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5. Test tubes for dilution of patient samples and reagents A and B.

6. Absorbent Paper for blotting the microplate wells.

7. Plastic wrap or microplate cover for incubation steps.

8. Vacuum aspirator (optional) for wash steps.


5.0 MATERIALS

Materials Provided:

A. Anti-TPO Calibrators – 1.0 ml/vial -Icons A-F

Samples of human serum, prepared in a special extended storage buffer and solution, that is stable at 2-8°C for 30 days. Store at 2-8°C.

C. Anti-TPO Tracer Reagent – 13ml/vial - Icon C

One (1) vial of anti-human IgG horse serum peroxidase (HRP) conjugate in a buffered matrix. A preservative has been added. Store at 2-8°C.

D. Light Reaction Wells – 96 wells – Icon U

One 96-well white microplate coated with streptavidin and preservative in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent Concentrate – 20ml/vial – No Icon

One (1) vial containing buffer salts and a dye. Store at 2-8°C (see Reagent Preparation Section).

F. Wash Solution Concentrate – 20ml/vial - Icon C

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C (see Reagent Preparation Section).

G. Signal Reagent A – 7ml/vial - Icon C

One (1) vial containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

H. Signal Reagent B – 7ml/vial - Icon C

One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or heparinised plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established performance, the serum specimen should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anticoagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the specimen(s) should be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay and establish reference intervals for clinically relevant controls to monitor assay performance. The reference intervals should be established using methodologies and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the specimens. All appropriate standard and additional methods should be used. The analytical procedures and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the specimens. All appropriate standard and additional methods should be used.

12. Light density intensities should be consistent with past experience. Significant deviation from established performance can indicate specimen instability. These controls should be treated as unknowns and values for clinically relevant controls to monitor assay performance.

13. Microplate luminometer for at least 0.2 seconds/ well.

14. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

8.0 REAGENT PREPARATION

1. Serum Diluent

Dilute the serum diluent concentrate to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.

2. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted wash buffer at room temperature 2-30°C.

3. Working Signal Reagent Solution - Store at 2 - 8°C. Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made) discard the unused solution if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of one (1) Signal Reagent B into Signal Reagent A and label accordingly.

4. Patient Sample Dilution (1/100)

Dispense 0.100ml (10µl) of each patient specimen into each ml serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note: Do not use reagents if they are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

Test procedure should be performed by a skilled individual or qualified professional.

1. Assemble the microwell strips for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the sealed pack before re-use. The absorbent paper.

2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the microplate.

3. Add 0.100ml (100µl) of TPO Biotinylated Conjugate Solution.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 30 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration, blot the plate dry with absorbent paper.

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) to each well, wait 1 minute or more.

8. Decant and wash the sample(s) (see Reagent Preparation Section).

9. Add 0.100ml (100µl) of anti-TPO Tracer Reagent to all wells.

10. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

11. Discard the contents of the microplate by decantation or aspiration, blot the plate dry with absorbent paper.

12. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) to each well and aspirate.

13. Wash the sample(s) (see Reagent Preparation Section).

14. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

15. Incubate for thirty (30) minutes at room temperature.

16. Discard the contents of the microplate by decantation or aspiration, blot the plate dry with absorbent paper.

17. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) to each well and aspirate.

18. Discard the sample(s) (see Reagent Preparation Section).

19. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

20. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the specimen(s) should be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

21. Microplate luminometer for at least 0.2 seconds/ well.

22. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.
10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-TPO (x-TPO) in unknown specimens. In the following example, the average RLU's (78755) of the unknown intersects the calibration curve at (316 IU/ml) x-TPO concentration (See Figure 1).

Note: Computer data reduction software designed for CLIA may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

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</table>

FIGURE 1

Mean RLU's for each duplicate serum reference

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the Anti-TPO AccuLite® CLIA test system. The number (n), mean (x) and standard deviation (σ) are given in Table 1. Values in excess of 400 IU/ml are considered positive for the presence of anti-TPO antibodies.

| Table 1 |
|------------------|--------|--------|--------|
| Expected Values for Anti-TPO AccuLite® CLIA Test System (in IU/ml) |
| Number (n) | Mean (x) | Standard deviation (σ) |
| Pool 1 | 20 | 9.1 | 0.8 |
| Pool 2 | 20 | 55.7 | 3.9 |
| Pool 3 | 20 | 121.8 | 7.5 |

Note: For re-assaying specimens with concentrations greater than 500 IU/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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Anti-TPO AccuLite® CLIA test system were determined by analyses on three different levels of pooled control sera. The number, mean, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

| Table 2 |
|------------------|--------|--------|--------|
| Within Assay Precision (Values in IU/ml) |
| Sample | N | X | σ |
| Pool 1 | 20 | 9.1 | 0.8 |
| Pool 2 | 20 | 55.7 | 3.9 |
| Pool 3 | 20 | 121.8 | 7.5 |

Note: For re-assaying specimens with concentrations greater than 500 IU/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.

14.2 Sensitivity

The Anti-TPO AccuLite® CLIA test system has a sensitivity of 1.0 IU/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Anti-TPO AccuLite® CLIA test system was compared with a reference method. Biological specimens from normal and disease state populations were used. The disease states included Hashemoto’s thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 125. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

| Table 4 |
|------------------|--------|--------|--------|
| Accuracy of the Anti-TPO AccuLite® CLIA test system in comparison with the reference method |
| Method | Mean | Least Square Regression Analysis | Correlation Coefficient |
| This Method | 185.3 | y = 1.02x – 6.5 | 0.985 |
| Reference | 192.3 | |

*Only slight amounts of bias between this test and the reference method are indicated by the closeness of the mean values. The