3.0 PRINCIPLE

A Sandwich Sequential CLIA Method (Type 1)

The reagents required for the sequential CLIA assay include immobilized antigen, circulating auto-antibody and enzyme-linked species specific antibody. A stored antigen, the immobilized antigen, takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and biotinylated Tg. In addition, the results are quantitated by a luminometer, which eliminates subjective subclinical levels of antibodies to Tg. In addition, the results sensitivity of chemiluminescence permits the detectability of 2.0 SUMMARY AND EXPLANATION OF THE TEST

Chemiluminescence.

Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Autoantibodies to Tg are often present in patients with autoimmune thyroid disease. Approximately 10 percent of healthy individuals have autoantibodies to Tg at low levels; higher concentrations are found in 30% and 85% of patients with Graves’ disease and Hashimoto’s thyroiditis, respectively. Antibodies to thyroid peroxidase (TPO) occur more frequently than autoantibodies to Tg in these conditions thus rendering anti-Tg assays are of little practical use. However, anti-Tg assays are useful while determining Tg levels in patients with thyroid conditions. The presence of Tg autoantibodies produces false results in determination of Tg levels both by competitive assays and by sandwich immunoassays.

Monobind’s microplate chemiluminescence immunoassay methodology provides the technique with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a micro-plate well. Biotinylated thyroglobulin (Tg) is added, then the reagents are mixed. Reaction results between the auto-antibodies to Tg and the biotinylated Tg to form an immune complex, which is deposited to the surface of coated wells through the high affinity reaction of biotin and streptavidin. After the completion of the required incubation period, aspiration or decantation separates the reagents that are not attached to the wells. An anti-human IgG-enzyme conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined in reaction with substrate to produce light (luminescence).

The employment of several serum references of known antibody activity permits construction of a dose response curve (graph) of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen’s antibody activity can be correlated with auto-immune antibody level. The intensity of light is directly proportional to the concentration of anti-thyroglobulin antibody in the specimen.

3.1 Materials Provided:

A. Anti-Tg Calibrators – 1.0 ml/vial Icons A/F
Six (6) vials of reference standards for anti-Tg at levels of 0(A), 50(B), 125(C), 500(D), 1000(E), and 2000(F) IU/mL. Store at 2-8°C. A preservative has been added.

B. Sample Diluent Concentrate – 1x concentrate
Dilute contents of Diluent Concentrate to 500ml with distilled or deionized water.

C. Wash Buffer
Dilute the serum diluent concentrate to 200ml in a suitable container (avoiding air bubbles) to dispense the wash.

D. Signal Reagent – 7.0ml/vial Icon C
One (1) vial containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

G. Signal Reagent A – 7.0ml/vial Icon C
One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

I. Microplate Luminometer

J. 96 Well Microplate - Icon C

K. Test tubes for dilution of patient samples and signal A/B. Each control kit contains four (4) tubes.

L. Vacuum aspirator (optional) for wash steps.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in tye 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 anticoagulant. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high blood doses (i.e. >5mg/day), serum samples should be collected at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. Specimens can not be assayed within this time. The samples(e) may be stored at temperatures of -20°C for up to 30 days. The vial is not to be freeze-thawed. Avoid repetitive freeze and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every assay for this purpose. Control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or assay error. Additional controls should be used to determine the reason for the variations.

Note: Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PREPARATION

Before proceeding with the assay, bring all reagents, sera and controls to room temperature. **Test procedure should be performed by a skilled individual or trained professional**! For multiple wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) directly to each well. Allow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, squeeze bottle should be disposed into a laboratory container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times. Do not shake the plate after enzyme addition.

12. Add 0.100ml (100µl) of anti-Tg Tracer/Signal Reagent to each well. Always add reagents in the same order to minimize reaction time differences between wells.

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

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

19. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) directly to each well. Allow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, squeeze bottle should be disposed into a laboratory container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.

21. Incubate at room temperature for five (5) minutes in the dark.

23. Read the RLUs (Relative Light Units) in each well in a microplate luminometer for a 0.2 seconds well. The results can be read within 30 minutes of adding the signal solution.

Note: For re-assaying specimens with concentrations greater than 2000 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.
10.9 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-Tg (x-Tg) in unknown specimens.

1. Record the RLUs obtained from the printout of the microplate reader as outlined in sample 1.
2. Plot the mean RLUs for each duplicate serum reference versus the corresponding x-Tg activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of x-Tg for an unknown, locate the average RLUs of the unknown intensity on the calibration curve at 1403 IU/ml x-Tg concentration (See Figure 1)*.

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

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Plotting of samples that should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated sera should be excluded.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of a reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis - for this and other devices, made by Monobind, can be requested via email from info@monobind.com.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. Heterophile antibodies: a problem for all immunosassays. Clin. Chem. 1988;34:273-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be performed by the various instruments that can be used to measure light output.

13.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

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

14.2 Sensitivity

This procedure has a sensitivity of 1.95 IU/ml. The sensitivity was ascertained by determining the variability of the 0 IU/ml calibrator using the 2σ (60% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The Anti-Tg AccuLite® CLIA test system was compared with two different immunoassays from different manufacturers, 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 specimens was 122. The least square regression equation and the correlation coefficient were computed for all paired test results in comparison with the reference method. The data obtained is displayed in Table 4.

14.4 Specificity

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

15.0 REFERENCES


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mRNA replication and spurious results.

* As measured in ten experiments in duplicate.