8. REAGENT PREPARATION

1. Serum Diluent
   Dilute the serum diluent to 200mL 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. Diluted buffer can be stored at 2-8°C for up to 60 days.

3. Working Substrate Solution
   Pour the contents of the vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C for a maximum period of 48 hours.

4. Patient Sample Dilution (1/100)
   Dispense 0.010mL (1.0µl) of each patient specimen into 1mL of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9. TEST PROCEDURE

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

"Test procedure should be performed by a skilled individual or trained professional!"

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate.
2. Replace any unused microwell strips back into the aluminum foil bag, seal and store at 2-8°C.
3. Pipette 0.050mL (50µl) of the appropriate serum reference, control or diluted patient specimen into the assigned well.
4. Add 0.100mL (100µl) of Tg Biotin Reagent.
5. Swirl the microplate gently for 20-30 seconds to mix and cover.
6. Incubate at room temperature for fifteen (15) minutes.
7. Add 0.050mL (50µl) of Stop Solution to each well and mix gently for 15-20 seconds.
8. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader.
9. Calculate the antibody level.
10. Quality control materials.

5.0 PRECAUTIONS
For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1 & 2 and HCV Antibodies. FDA licensed, known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially infectious material. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, “Hospital Infection Control Practices Advisory Committee (HICPAC).” 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMENT COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type. The final preparation of venipuncture samples should be observed. For accurate comparison to established normal (IgG) range reporting serum samples should be collected. The blood should be collected in a plain redtop venipuncture tube without additives or anticoagulants (for serum) or evacuated tubes containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the collection of venipuncture samples should be observed. For accurate comparison to established normal (IgG) range reporting serum samples should be collected. The blood should be collected in a plain redtop venipuncture tube without additives or anticoagulants (for serum) or evacuated tubes containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Calculations and results should be read within thirty (30) minutes of adding the stop solution.
10.0 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 IU/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 intersection point on the curve, and read the concentration (in IU/ml) 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 IU/ml anti-Tg concentration (see Figure 1).

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

11.0 QC. 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.

12.0 RISK ANALYSIS

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

12.1 Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly hemolyzed or grossly contaminated samples should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of the stop solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, this incubation step should be added in the same sequence to eliminate any time-variation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation step(s) may result in poor replication and spuriously results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. 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 a between assay precision of over 3.0 units of absorbance.
10. Samples, which are contaminated microbiologically, should not be used.
11. Accurate and precise pipetting, as well as following the exact pipetting procedure should be used.

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. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If tests are altered, such as by mixing parts of procedures, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. It is important to calibrate all the equipment e.g. Pipetters, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
6. Analysis as required by CE Mark (IVD Directive 98/79/EC), for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.3 Accuracy

The anti-Tg AccuBind™ ELISA has a sensitivity of 1.94 IU/ml. The sensitivity was ascertained by determining the variability of the ‘0 IU/ml’ calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

12.4 Specificity

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

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the Anti-Tg AccuBind™ test system. The number (n) mean (X), standard deviation (σ) and coefficient of variation (CV) for each of these control sera are presented in Table 2 and Table 3.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the anti-Tg AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean (X), X̄ value (X̄), and σ value (σ) are shown in Table 2. The sensitivity was ascertained by determining the variability of the ‘0 IU/ml’ calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

15.0 REFERENCES


Revision: Date: 060712 DCO: 0653 Cat #: 1025-300

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CE & FDA

EC REP

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†Based as measured in ten experiments in duplicate.