**1.0 INTRODUCTION**

Tg determination has been done with various methods using a replacement for it. Assessment of Tg levels aids in management of the disease. Tg levels are found to be normal in patients with metastatic thyroid carcinoma. Serial measurements of Tg is most useful in detecting recurrence of differentiated thyroid carcinoma.

**2.0 SUMMARY AND EXPLANATION OF THE TEST**

Human thyrogbulin (Tg) is a large glycoprotein (660 KD) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intrathyroid synthesis of primary thyroid hormones like Triiodothyronine (T3) and Thyroxine (T4).

Tg is elevated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto’s thyroiditis and Graves Disease.

Tg levels are found to be normal in patients with inactive thyroid gland and the antibody used are not associated with the antigen.

**3.0 PRINCIPLE**

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 Time of the specimen is ascertained by centrifuging 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 sample(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 12 months. Avoid repeated freezing and thawing. When assayed in duplicate, 0.100 ml (100µl) of the specimen is required.

**4.0 REAGENTS**

Materials Provided:

- A. Thyroglobulin Calibrators – 1.0 ml/vial – Icons A - F
- B. x-Tg Biotin Reagent – 13ml/vial – Icon
- C. Kit and component stability are identified on the

- 1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml
- 2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls
- 3. Paintbrush
- 4. Swirl the microplate gently for 20-30 seconds to mix.

**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 reference tests.

**6.0 SPECIMENT 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.

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**7.0 QUALITY CONTROL**

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These data are then used to optimize assay performance determined in every test procedure performed. Quality control charts should be maintained to examine in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

**8.0 REAGENT PREPARATION**

1. Wash Buffer
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Incubate for 2 hours at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container (Avoiding air bubbles). Discard the wash and repeat two (2) additional times.
8. Add 0.050ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
9. Add 0.100 ml (100µl) of the x-Tg Biotin Reagent to all wells
10. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container (Avoiding air bubbles). Discard the wash and repeat two (2) additional times.
12. Add 0.100 ml (100µl) of substrate to all wells. Always add reagents within the order to minimize reaction time differences between wells.

**9.1 ALTERNATE PROCEDURE (Time 2hr 15min)**

This procedure can be used with the help of a laboratory hematology shaker.

1. Format the microplates wells for each calibrator, control and patient sample to be assayed in duplicate.
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) labeled monoclonal antibody to each well. It is very important to dispense all reagents close to the bottom of the microwell and swirl to mix.
4. Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container (Avoiding air bubbles). Discard the wash and repeat two (2) additional times.
7. Add 0.100 ml (100µl) of x-Tg Biotin Reagent to all wells
8. Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
10. Submit the appropriate calibrator, controls and samples into the assigned wells.

**10. CALCULATION OF RESULTS**

A dose response curve is used to ascertain the concentration of human thyroglobulin (Tg) in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader for the corresponding Tg concentration.
2. Plot the absorbance for each duplicate reference versus the corresponding Tg concentration on a linear graph.


3. Mayo Medical Laboratories: test Catalog, Rochester, MN


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