1. INTRODUCTION

The Quantitative Determination of Thyrotropin Concentration in Human Serum by a Micropete Enzyme Immunoassay, Chemiluminescence.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 Daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator for the diagnosis of primary and secondary (plutitary) hypothyroidism. The structure of human TSH is similar to that of the pituitary and placental gonadotropins, consisting of two identical α-subunits and one identical β-subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with a similar or identical mechanism to the enzyme. The TSH molecule has a linear structure of the protein core with carbohydrate side chains; the latter accounts for 10% of the molecular weight.

2.1 PRINCIPLE

Immunoenzymometric assay (Type 3)

The essential requirement for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in each case a non-native antigen. This assay immobilizes TSH on the surface of the microwell through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steadic to block, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[ \text{Ab}^{\text{m}} \cdot \text{Ab} \cdot \text{TSH} \]

In increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of TSH, is an early and sensitive indicator of hypothyroidism and is a notable phenomenon present on the surface of these and other autoimmunological antibodies.

Additionally, TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduce the response of the pituitary to the stimulatory effects of TRH. In secondary hypothyroidism, the pituitary to the stimulatory effects of TRH. In secondary hypothyroidism, concentrations of T4 are usually low and less than those expected for normal pituitary function. Increase levels of T3 and T4 reduce the response of the pituitary to the stimulatory effects of TRH. In secondary hypothyroidism, concentrations of T4 are usually low and less than those expected for normal pituitary function.

4.0 REAGENTS

Materials Provided:

- A. TSH Calibrators – 1 ml/vial – Icons A-G
- Seven (7) vials of references for TSH Antigen at levels of 0.1, 0.5 (B), 2.5 (C), 5.0 (D), 10 (E), 20 (F) and 40 (G) µIU/ml. Antigen level is labeled as “µIU/ml”.
- B. TSH Tracer Reagent – 13 ml/vial – Icon C
- One (1) vial containing enzyme labeled antibodies (Enzyme labeled antibodies for TSH) in buffer, and preserved, Store at 2-8°C.
- C. Light Reaction Wells – 96 wells – Icon U
- One (1) vial containing hydrogen peroxide (H2O2) in buffer, and preserved, Store at 2-8°C.
- D. Wash Solution Concentrate – 20 ml/vial – Icon A
- One (1) vial containing surfactant in Buffer. Preserved has been added. Store at 2-8°C.
- E. Signal Reagents A – 7 ml/vial – Icon C
- One (1) vial containing luminol in buffer, Store at 2-8°C.
- F. Signal Reagent B – 7 ml/vial – Icon C
- One (1) vial containing hydrogen peroxide (H2O2) in buffer, Store at 2-8°C.
- G. Package Insert

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

Note 2: Avoid extended exposure to heat and light. Opened reagents are to be stored 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:

- Pipette(s) capable of delivering 0.050 and 100ml (50 & 100µl) volumes with a precision of better than 1.5%.
- Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the well.
- Add 2 ml of a and 1ml of b per two (2) eight well strips. Stir each appropriately and use a microplate reader to measure the absorbance at 405 nm and 492 nm. If adequate sample is not used within 30 minutes after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of the Signal Reagent B into Signal Reagent A and label accordingly.

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

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20 ± 2°C). 

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

1. Format the microwells’ plates for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag and store at 2-8°C.

2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the well.

3. Add 2 ml of a and 1ml of b per two (2) eight well strips. Stir each appropriately and use a microplate reader to measure the absorbance at 405 nm and 492 nm. If adequate sample is not used within 30 minutes after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of the Signal Reagent B into Signal Reagent A and label accordingly.

4. Swirl the microplate gently for 20-30 seconds and mix the microplate gently for 30 seconds. Incubate 45 minutes at room temperature.

5. Discard the contents of Wash Concentrate for decantation or aspirate. If decanting, tap the plate dry with absorbent paper. Store at 2-8°C.

6. Add 0.5 ml of b (50µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times. The automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, wash 1-2 times. Rinse with distilled or deionized water in a suitable storage container (avoiding air bubbles) to dispense the wash. Discard the wash and repeat four (4) additional times.

7. Add 0.5 ml of c (350µl of wash buffer) to each well. The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be followed. It is very important to dispense all reagents close to the surface of each well. Do not allow the specimen to separate the serum from the cells.

8. Do not disturb the microwell format. Mix gently for 30 seconds. Repeat the washing steps for five times (5) days. If the sample(s) can be assayed within this time frame, may 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, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality assurance tests shall be performed to ascertain the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that may affect the results include the use of the dose response curve for run-to-run reproducibility. In addition, maximum light intensity should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reagents for the next omissions.

9.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of TSH in unknown specimens. Record the RLUs obtained from the printout of the microplate reader.

Plot the RLUs for each duplicate serum reference calibrator, control or specimen to separate the serum from the cells.

10.0 REAGENT PREPARATION

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

10.1 Pipette 0.5 ml of a (50µl) of Reagents (light units) in each well in a microplate luminescent for at least 0.2seconds per well. The results may be read within 30 minutes of adding the signal solution.
In order for the assay results to be considered valid the Q.C. PARAMETERS should be met.
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 lipemic, hemolysed or grossly contaminated specimen(s) should not be used.
4. If more than one plate is used, it is recommended to repeat the dose response curve.

The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLU’s for the G calibrator only and may be used in lieu of a dose response curve.

It is important to keep in mind that expected values for normal population are dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each method should be compared against the expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population that represents the area in which the laboratory is located.

### 14.0 PERFORMANCE CHARACTERISTICS

#### 14.1 Precision

The within assay precision of the TSH AccuLite® CLIA Test System were determined by analyses on three different levels of pool control sera. The mean number, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

#### 14.2 Interpretation

Measurements and interpretation of results must be performed by a skilled individual or trained professional.

1. If more than one plate is used, it is recommended to repeat the dose response curve.
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