Section 3: Thyroid Tests for the Clinical Biochemist and Physician

A. Total Thyroxine (TT4) and Total Triiodothyronine (TT3) Methods

Thyroxine (T4) is the principal hormone secreted by the thyroid gland. All the T4 in the circulation is derived from thyroidal secretion. In contrast, only about 20% of circulating triiodothyronine (T3) is of thyroidal origin. Most of the T3 in blood is produced enzymatically in nonthyroidal tissues by 5'-monodeiodination of T4 *(121)*. In fact, T4 appears to function as a pro-hormone for the production of the more biologically active form of thyroid hormone, T3. In the circulation, most (~99.98%) T4 is bound to specific plasma proteins, thyroxine-binding globulin (TBG) (60-75%), TTR/TBPA (prealbumin/transthyretin) (15-30%) and albumin (~10%) *(12,16)*. Approximately 99.7% of T3 in the circulation is bound to plasma proteins, specifically TBG. This represents a 10-fold weaker protein-binding than seen for T4 *(12)*. Protein-bound thyroid hormones do not enter cells and are thus considered to be biologically inert and function as storage reservoirs for circulating thyroid hormone. In contrast, the minute free hormone fractions readily enter cells by specific membrane transport mechanisms to exert their biological effects. In the pituitary, the negative feedback of thyroid hormone on TSH secretion is mediated primarily by T3 that is produced at the site from the free T4 entering the thyrotroph cells.

Technically, it has been easier to develop methods to measure the total (free + protein-bound) thyroid hormone concentrations, as compared with tests that estimate the minute free hormone concentrations. This is because total hormone concentrations (TT4 and TT3) are measured at nanomolar levels whereas free hormone concentrations (FT4 and FT3) are measured in the picomole range and to be valid, must be free from interference by the much higher total hormone concentration.

1. Methods for measuring Total Thyroid Hormones

Serum total T4 and total T3 methods (TT4 and TT3) have evolved through a variety of technologies over the past four decades. The PBI tests of the 1950s that estimated the TT4 concentration as "protein-bound iodide" were replaced in the 1960s first by competitive protein binding methods and later in the 1970s by radioimmunoassay (RIA) methods. Currently, serum TT4 and TT3 concentrations are measured by competitive immunoassay methods that are now mostly non-isotopic and use enzymes, fluorescence or chemiluminescence molecules as signals *(135)*. Total hormone assays necessitate the inclusion of an inhibitor (displacing or blocking agent) such as 8-anilino-1-napthalene-sulphonic acid (ANS), or salicylate to release the hormone from binding proteins *(136)*. The displacement of hormone binding from serum proteins by such agents, together with the large sample dilution employed in modern assays, facilitates the binding of hormone to the antibody reagent. The ten-fold lower TT3 concentration, as compared with TT4 in blood, presents both a technical sensitivity and precision challenge despite the use of a higher specimen volume *(137)*. Although reliable high-range TT3 measurement is critical for diagnosing hyperthyroidism, reliable normal-range measurement is also important for adjusting antithyroid drug dosage and detecting hyperthyroidism in sick hospitalized patients, in whom a paradoxically normal T3 value may indicate hyperthyroidism.

Despite the availability of highly purified preparations of crystalline L-thyroxine and L-triiodothyronine (i.e. from the United States Pharmacopoeia (16201 Twinbrook Parkway, Rockville, MD 20852) no TT4 or TT3 reference methods have yet been established *(138,139)*. Further, the hygroscopic nature of the crystalline preparations can affect the accuracy of gravimetric weighing *(140)*. Secondly, the diluents used to reconstitute L-T4 and L-T3 preparations for use as calibrators are either modified protein matrices or human serum pools that have been stripped of hormone by various means. In either case, the protein composition of the matrix used for the calibrators is not identical to patient sera. This can result in the protein binding inhibitor reagent (e.g. ANS) releasing different quantities of hormone from calibrator matrix proteins than from the TBG in patient specimens. This may impact the diagnostic accuracy of testing when the binding proteins are abnormal, such as in NTI.

Guideline 9. For Manufacturers Developing TT4 and TT3 Methods

Method biases should be reduced by:

- The development of L-T4 and L-T3 reference preparations and establishing international reference methods.
- Ensuring that instruments are not sensitive to differences between human serum and the calibrator matrix.
- Ensuring that during the test process, the amount of thyroid hormone released from serum binding proteins is the same as that released in the presence of the calibrator diluent.

2. Diagnostic Accuracy of Total Hormone Measurements

The diagnostic accuracy of total thyroid hormone measurements would equal that of free hormone if all patients had identical levels of binding proteins (TBG, TTR/TBPA and albumin) with similar affinities for thyroid hormones. Unfortunately, abnormal serum TT4 and TT3 concentrations are more commonly encountered as a result of binding protein abnormalities than result from true thyroid dysfunction. Patients with serum TBG abnormalities secondary to pregnancy or estrogen therapy, as well as genetic abnormalities in binding proteins, are frequently encountered in clinical practice *(141)*. Abnormal TBG concentrations and/or affinity for thyroid hormone can distort the relationship between total and free hormone measurements *(142)*. Additionally, some patient sera contain other abnormal binding proteins such as autoantibodies to thyroid hormones that render total hormone measurements diagnostically unreliable *(143-145)*. These binding protein abnormalities compromise the use of TT4 and TT3 measurements as stand-alone thyroid tests. Instead, serum TT4 and TT3 measurements are typically made as part of a two-test panel that includes an assessment of binding protein status, made either directly by TBG immunoassay or by an "uptake" test [Section-3 B2(b)]. Specifically, a mathematical relationship between the total hormone concentration and the "uptake" result is used as a free hormone "index" *(146)*. Free hormone indices (FT4I and FT3I) have been used as free hormone estimate tests for three decades but are rapidly being replaced by one-test free hormone estimate immunoassays [Section-3 B3].

3. Serum TT4 and TT3 Normal Reference Intervals

Serum TT4 values vary between methods to some extent. Typical reference ranges approximate 58 - 160 nmol/L (4.5-12.6 µg/dL). Likewise, serum TT3 values are method dependent, with reference ranges approximating 1.2 - 2.7 nmol/L (80 –180 ng/dL).

Guideline 10. Serum Total T4 (TT4) and Total T3 (TT3) Measurements

Abnormal serum TT4 and TT3 concentrations are more commonly encountered as a result of binding protein abnormalities and <u>not</u> thyroid dysfunction.

- Free T4 estimate tests (FT4) are preferred over TT4 measurement when TBG concentration is abnormal. However, FT4 tests but may be diagnostically inaccurate when the affinity of TBG is altered or abnormal T4-binding proteins are present.
- Total hormone assays (TT4 and TT3) should remain readily available to evaluate discordant free hormone tests.