

Laboratory Tests of Thyroid Function: Uses and Limitations

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Laboratory tests are the most commonly used aids in the diagnosis and monitoring of individuals who have thyroid disease. In general, laboratory tests for thyroid evaluation are similar in accuracy and reliability to other laboratory tests. In some situations, thyroid tests can provide misleading or inaccurate information because, for the most part, test methods used in clinical laboratories do not use “definitive” methods for measuring compounds but instead use “comparative” methods. In almost all cases, solutions containing known amounts of the compounds of interest are analyzed, and some physical parameter of the solution (eg, amount of light absorbed) is measured. Samples from patients are then measured using the same method, and the signal obtained is compared with that from the known samples. This requires the assumption that the known and patient samples are similar in all other respects, save for the amount of the compound of interest. When this assumption is not valid, then the results are affected.

This article briefly summarizes the common methods of laboratory testing relating to thyroid disease and discusses specific information for individual tests on methods of analysis, their limitations, and situations where caution should be used in interpreting the results of thyroid tests. For a number of tests, the degree of inaccuracy varies with the specific method used in a given laboratory. Because laboratories generally do not have information on the clinical status of or medications taken by the patient at the time of testing, it is difficult for the laboratory to recognize when they are dealing with a sample that may produce misleading results. It is important for endocrinologists to be familiar with the methods used in the laboratories that they use and to contact the laboratory when test results seem to be

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misleading. In many cases, the laboratory can perform additional tests or evaluate the sample for interferences.

Principles of laboratory testing used for thyroid testing

To measure the small amounts of thyroid hormones and peptides, simple chemical analyses generally do not have adequate sensitivity. Most thyroid tests are performed using one of two formats of immunoassay (Table 1). For thyroid hormones (and in one available assay for thyroglobulin), competitive immunoassays are used. In this format, a limited amount of antibody to thyroid hormone is incubated with the patient’s serum, and a known amount of the same thyroid hormone is labeled. The labeled hormone and unlabeled hormone compete for binding by the antibody. The amount of label bound to the antibody is inversely proportional to the amount of hormone in the patient’s serum. Competitive immunoassays are subject to interference by compounds that are similar in structure to the compound of interest; this is generally not a problem for thyroid hormones or thyroglobulin. Patients who have antibodies to the compound of interest (a common issue in thyroglobulin assays [1] and an occasional problem with thyroid hormone assays [2]) often have falsely increased results: As some of the labeled hormone becomes bound to the antibody in the serum, less is available to bind to the testing antibody, which leads to the false impression of high hormone levels.

A second format of immunoassay, often referred to as immunometric assay, is most commonly used to measure peptide hormones, such as thyroid-stimulating hormone (TSH), calcitonin, and thyroglobulin. In this format, a large amount of antibody to the hormone is bound to a solid support, and patient serum is added. After incubation, the support is washed to remove residual serum, and a second labeled antibody to the hormone is added. (Such assays are sometimes called “sandwich” assays; the two

Table 1
Features of immunoassay formats

Feature	Competitive	Sandwich
Most used for	Small molecules (T3, T4)	Larger molecules (TSH, calcitonin, thyroglobulin)
	Older assays for TSH, calcitonin, thyroglobulin	
Interferences by closely related compounds	Yes, significant	Rarely
Interferences by antibodies to compounds	Falsely increased	Usually decreased, rarely increased
Interferences by heterophile antibodies	Rare	Affects 0.1%–1% of samples

Abbreviation: TSH, thyroid-stimulating hormone.

antibodies represent the bread, and the hormone is the “filling” in the sandwich.) When the solid support is washed, the amount of labeled antibody remaining on the support should be directly proportional to the amount of hormone in serum.

Immunometric assays are generally much more specific for hormones than competitive assays; the use of two different antibodies, often reacting with different parts of the hormone, reduces the likelihood of interference from chemically similar compounds. There are other causes of interference in immunometric assays. Antibodies to the hormone can produce falsely low results with immunometric assays, a common problem in thyroglobulin measurement [3]. Human antibodies to the antibodies used in the assays may lead to attachment of the indicator antibody to the solid support, even in the absence of the hormone. This most commonly occurs with human antibodies to mouse proteins because mouse monoclonal antibodies are most widely used, although other antibodies that react with a variety of animal proteins (ie, “heterophile” antibodies) may also occur [4,5]. Rheumatoid factor is also a potential interference in immunometric assays. Although assay manufacturers add substances to reduce the potential for interference [6], it has been estimated that one to two samples per thousand have high enough antibody titers to cause falsely increased results [7]. This is most problematic for hormones that should be in low concentrations, such as thyroglobulin in a thyroidectomized patient or TSH in a hyperthyroid patient. Several approaches can be used when such interferences are suspected [8]. Commercial neutralizing reagents with extremely high amounts of mouse immunoglobulin can be preincubated with the patient serum. Serial dilution of the sample can document that the expected fall in results does not occur. Repeating the assay with a kit from a different manufacturer often shows significant differences in results because different assays have differing susceptibility to interference. When the results are incompatible with the clinical setting, the endocrinologist should contact the laboratory to ask whether the laboratory checked for such interferences and if not to request that one or more of the above steps be taken to evaluate for antibody interference.

Specific thyroid tests

Thyrotropin

Measurement of TSH has become the principal test for the evaluation of thyroid function in most circumstances. Assays for TSH have been classified in generations, based on the functional sensitivity of the assay (ie, the level at which the repeatability of measurement is $\pm 20\%$). The National Academy of Clinical Biochemistry (NACB) guidelines recommend that laboratories use assays with third-generation functional sensitivity (0.02 mU/L) or better [9].

An unresolved issue is the upper limit of “normal” for TSH. Laboratories typically establish reference limits based on the central 95% of values seen in apparently healthy individuals; for TSH, such limits typically are around 0.4 to 0.5 mU/L for the lower limit and 4.5 to 5.5 mU/L for the upper limit. This approach has been called into question for several reasons. First, prospective studies of thyroid function have found that risk of subsequent hypothyroidism rises at TSH values higher than 2.0 mU/L [10]. Higher TSH values (within traditional reference limits) are typically found in persons who have risk factors for future thyroid disease, such as family history, presence of thyroid antibodies, pregnancy, or use of drugs affecting thyroid function (eg, amiodarone) [11,12]. This has led some investigators, including the authors of the NACB guidelines [9], to suggest that upper reference limits for TSH should be lowered to 2.5 mU/L. Not all individuals who have TSH values that are higher than this cutoff (or even those who have slightly higher values) develop overt hypothyroidism in long-term follow-up. Evaluation of clinical outcomes has suggested that treatment of subclinical hypothyroidism is not warranted until TSH exceeds 10 mU/L [12]. There thus seems to be a range of TSH values for which an alternative term, such as “borderline” or “at risk,” might be more appropriate than the traditional interpretation of all values outside the reference limits as “abnormal.”

TSH measurements have less-than-ideal agreement between different assays, which limits the ability to define arbitrary reference limits that should be used by all laboratories. For example, in a recent survey involving 2580 patients, the mean values for TSH using different assays, on a sample with “normal” TSH, ranged from 1.24 to 1.73 mU/L [13]. Because different manufacturers’ assays use antibodies that recognize different epitopes of TSH and because multiple different forms of TSH are present in the circulation, it is difficult to reach agreement between assays. It is likely that, for use of a single common value for the upper reference limits, a process similar to the glycohemoglobin standardization program is required.

A number of conditions affect TSH or its measurement and may cause discordance between TSH levels and the clinical picture of the patient (Table 2). In acutely ill individuals, the range of TSH values seen in apparently euthyroid individuals changes markedly. A number of factors, such as cortisol, dopamine, and cytokines, affect TSH production. In one study of individuals subsequently shown to be euthyroid, 95% of TSH values were between 0.1 and 20 mU/L when the same individuals were acutely ill [14]. Only half of acutely ill individuals who had TSH values above 20 mU/L were found to have thyroid disease. A review of published studies found that TSH was predictive of thyroid disease only beyond these broadened “reference” values [15]. As a result, TSH levels must be interpreted with caution in hospitalized individuals unless values are below 0.1 or above 20 mU/L, and several groups have advised against routine testing of thyroid function in patients who have acute illness.

Table 2
Causes of misleading thyroid-stimulating hormone results

Condition	Effect
Acute illness	TSH central 95% reference interval widens to 0.1–20 mU/L
Central hypothyroidism	Abnormal forms predominate, with high immunoassay/bioactivity ratio, leading to falsely high results
Heterophile antibodies	Falsely increased results; results differ between assays, nonlinear on dilution Neutralized by nonimmune mouse serum for assays using mouse antibodies
TSH autoantibodies	Falsely increased results; results differ between assays, nonlinear on dilution Not neutralized by mouse serum Removed by polyethylene glycol precipitation

Abbreviation: TSH, thyroid-stimulating hormone.

Abnormal forms of TSH may predominate in individuals who have central hypothyroidism. Decreased sialylation of TSH is common in central hypothyroidism and results in reduced bioactivity and increased TSH half-life [16,17]. Because TSH is typically measured by immunoassay, it is important to consider the effects of these changes on measured TSH. The ratio of bioactive to immunoreactive TSH is markedly reduced in central hypothyroidism; this results in TSH values that are seldom low but that typically fall within the reference range and that, in about 15% of cases, are elevated [18]. With successful treatment, TSH values typically fall below the lower reference limit or become undetectable.

Measurement of TSH can be subject to interference from heterophile antibodies and rheumatoid factor, producing falsely increased results. This is most commonly problematic in hyperthyroid individuals, in whom nonsuppressed TSH suggests TSH-producing tumors or thyroid hormone resistance. Greater degrees of interference can result in persistent TSH elevation despite successful treatment of patients who have hypothyroidism. Rarely, autoantibodies to TSH may occur or may develop after injections of bovine TSH. In one study, clinically significant interference occurred in only 2 of 300,000 samples tested [19]. TSH autoantibodies can cross the placenta, leading to a false impression of neonatal hypothyroidism [20]. One clue to the presence of either type of interference is relatively stable TSH values despite changing thyroid hormone levels. Another clue is discrepant results between different laboratories using different TSH assays; in the baby who had TSH antibodies that crossed the placenta, TSH values varied between 4 and 213 mU/L with four different assays [20]. If such interference is suspected clinically, the laboratory should be contacted to determine if it has evaluated for interfering substances and to request evaluation if it has not been performed.

Total thyroxine and triiodothyronine

In the 1970s and 1980s, most tests of thyroid hormone production evaluated total thyroid hormone concentration. Because well over 99% of T4 and T3 is bound to binding proteins (thyroxine-binding globulin [TBG], albumin, and transthyretin, also termed thyroxine-binding prealbumin), total thyroid hormone levels are affected by changes in binding protein levels or binding affinity. Assays for total thyroid hormone are now less frequently used, although they are still available in many laboratories. The major reasons for the decreased popularity of total thyroid hormone assays are the effects of binding protein changes and the lower sensitivity of total hormone assays for early thyroid dysfunction. Total thyroid hormone levels are more often abnormal due to binding protein changes than to thyroid dysfunction [9].

Assays for total thyroxine have reasonable comparability between methods from different manufacturers. In a study of 1528 laboratories, differences between methods were less than 10% from the result for total T4 for all but two methods [13]. Repeatability of results over time was excellent in another study using two mailings of fresh frozen serum 6 months apart [21]. For total T3, results in 926 laboratories were more than 10% from the actual result for 58% of methods and were more than 20% from the actual result for 33% of methods [13].

Changes in levels of binding proteins, particularly increases in TBG, are relatively common. Frequent causes of abnormal TBG levels are summarized in Table 3. With abnormal TBG, total thyroxine (T4) and triiodothyronine (T3) are affected. In the less common familial dysalbuminemic hyperthyroxinemia, total T4 is typically increased, whereas total T3 is not. In general, when there is an isolated abnormality in TBG, total thyroid values, when corrected for TBG levels by the use of T-uptake tests, reflect a euthyroid state. When hypo- or hyperthyroidism coexists with abnormal binding proteins, calculated free thyroxine index is often inappropriately normal [22]. Drugs that affect the binding of hormones to protein can also affect total thyroid hormone measurements; such drug effects are discussed under free T4 measurements.

Table 3
Factors affecting thyroxine-binding globulin levels

Factor	Increased	Decreased
Drugs	Estrogens, fluorouracil, opiates, methadone, mitotane, tamoxifen	Androgens, danazol, glucocorticoids, nicotinic acid
Liver disease	Acute, chronic hepatitis	Cirrhosis
Congenital disorders	Not reported	Rare deficiency
Renal disease	None	Nephrotic syndrome
Other conditions	Pregnancy	Malnutrition

Multiple abnormalities related to thyroid hormone binding protein levels and changes in binding affinity are common in patients who have acute illness. Levels of all binding proteins decrease in acute illness, and the affinity constant for TBG binding is reduced, leading to a disproportionate decrease in total thyroid hormone levels relative to changes in free thyroid hormones. Free fatty acid competes with thyroid hormone for binding sites on albumin; free fatty acid levels are commonly increased in acute illness as well. The NACB guidelines recommend the use of total T4 levels in persons who have acute illness [9]. Although one study found estimated free thyroid hormone levels calculated from total T4 and T-uptake to be normal in most acutely ill individuals [23], two other studies showed significant limitations of total T4 in acute illness. In one study, the calculated free thyroxine index was low in about 10% to 20% of cases, independent of the assay used [22]. In another study, calculated thyroid index based on total T4 levels was found to correlate with the reduced levels of all three binding proteins [24]. Total and free T3 levels are also typically decreased in individuals who have acute illness, regardless of method used.

Another cause for erroneous total thyroid hormone levels is autoantibodies to thyroid hormones. These are most commonly found in persons who have the major autoimmune thyroid disorders, Graves' disease and Hashimoto's thyroiditis. A recent review estimates the prevalence of antithyroid hormone antibodies in these disorders to be approximately 10%, although not all cause significant interference in thyroid hormone assays [2]. Typically, total thyroid hormone levels are increased, sometimes markedly, by such autoantibodies. The degree of elevation varies between different methods, and increases may involve T4, T3, or both. Naturally occurring antibodies that react with the conjugate used in at least one assay are also capable of interfering in thyroid uptake assays, causing falsely low results [25].

Free thyroxine and triiodothyronine

Assays for measurement of free thyroxine have become the most common direct test of thyroid gland function, replacing total thyroid hormone measurements in many laboratories. A recent review highlights some of the problems in the measurement of free thyroxine [26]. The measurement of the extremely small relative amount of free thyroid hormone in the presence of massive amounts of protein-bound hormone must make a number of assumptions. The assay must be highly specific for the free form of the hormone and must not measure any of the bound hormone (or, if it does, it must measure the same proportion in all samples, as in the materials used to calibrate the assay). There must be no other substances that change the affinity of binding proteins for thyroid hormone compared with binding in the calibrators. The intrinsic affinity constants of the binding proteins must be similar to that in the calibrators. The method should be unaffected

by changes in the level of the binding proteins. A number of conditions exist that can affect these assumptions and thus the measurement of free thyroid hormones.

One problem with measurement of free thyroid hormones is that there is no definitive method to accurately measure only free thyroid hormone [27]. The definitive method for total thyroid hormone, isotope dilution mass spectrometry, has been applied, in combination with ultrafiltration, for the measurement of free thyroid hormone but has not been evaluated under a wide variety of conditions [28]. The gold standard comparative method, equilibrium dialysis, is known to produce higher results in persons who have acute illness [29] but is thought to provide reliable results under most other circumstances. Because there is no current definitive method, this assumption cannot be validated. The temperature at which the test is performed affects the equilibrium and the results [30], and drugs that alter binding protein affinity affect equilibrium dialysis results.

Few laboratories perform equilibrium dialysis, and most laboratories in North America and Europe typically use methods relying on one of two basic principles [31]. The first assays for free thyroid hormones to be widely available used analogs of thyroid hormone in a competitive format, so that the amount of analog bound to antibody would be inversely proportional to free thyroid hormone concentration. Analogs were originally small-molecular-weight compounds developed so that they did not bind to TBG; however, analogs bound to albumin to varying degrees. Most modern “analog” assays use labeled antibodies (in such assays, T3 is typically used to compete for binding sites on antibody with free T4 in the sample) or an analog fixed to a solid support that competes with free hormone in the sample for binding to a limited amount of a labeled antithyroid hormone antibody. Analog assays are typically used in assays from Bayer, Ortho, and Roche, which collectively are used by about half of laboratories [21]. The other common format uses a two-step approach. Samples are briefly incubated with a bound antibody to thyroid hormone; after washing, a labeled thyroid hormone is added to the bound antibody, and the amount of label remaining bound to the antibody after washing is inversely related to the amount of free thyroid hormone in the sample. Two-step methods are used in assays from Abbott, Beckman-Coulter, and Dade-Behring, and some assays from Diagnostics Production Corporation by the remaining half of laboratories.

The differences in methods lead to variability in results for the same sample between different laboratories. In 1744 laboratories performing free T4 measurement on a fresh frozen sample, different methods reported average free T4 results between 0.79 and 1.17 ng/dL; 38% of methods had average results that differed more than 10% from the average of all methods, and 8% had average results more than 20% different [13]. For most methods, results were comparable on two different mailings of the same sample 6 months apart [21]. For free T3, differences between methods were greater than those for free T4; 55% of methods had average results more than

10% from the mean of all methods, whereas 27% of methods differed from the average results by more than 20% [13].

A number of situations can lead to misleading results for free T4. Although less information is available, it is likely that similar changes occur with assays for free T3 because the assay principles are similar. Although free T4 methods are designed to be unaffected by changes in TBG, all (nonequilibrium dialysis) assays are affected to some degree by abnormal TBG levels [32]. In an elegant study using titrated, equilibrated amounts of free thyroxine, extremes of TBG concentration correlated directly with measured free T4. For a number of competitive analog-type and two-step methods, there was little effect of commonly encountered abnormal levels of TBG on measured free T4 [33].

Most studies evaluating free T4 assays in acute illness were done with older methods that commonly produced low results. A number of changes that occur with acute illness could theoretically affect free T4 measurement. Decreases in binding protein levels occur. Changes in pH can alter binding affinities. Free fatty acids increase in many acutely ill individuals, and these can displace T4 from albumin and can affect its measurement in unpredictable ways. In one recent study involving bone marrow transplant recipients, equilibrium dialysis and two one-step methods gave increased results in 20% to 40% of samples, whereas two other one-step methods had results that were mostly within the healthy normal range but with 10% to 20% below normal results; total T4 was normal in 95% of those studies [32]. Although data are limited, immune extraction results were less likely to be abnormal in acute illness than with other methods, with only 10% of results below the lower reference limit [29]. Normal results for free T4 can, therefore, be considered reliable, but abnormal results may be due to problems with the assay method. Laboratories should evaluate the effects of acute illness on their method, and, when requested, should use a different method (preferably one with an artifact that changes results in the opposite direction) to evaluate abnormal results. Discordant results (ie, low by one method and high by another) are likely to represent artifacts of measurement, whereas concordant results by methods with artifacts that change free T4 in opposite directions likely represent thyroid dysfunction.

A number of drugs alter the binding of thyroid hormone to one or more of its binding proteins [34]; these are summarized in Table 4. Most of these effects are method independent and as a result can cause abnormal free thyroid hormone levels in all laboratories.

Calcitonin

Calcitonin measurement has evolved over time, with measurement methods changing from simple competitive immunoassays to sandwich assays. Because of varying levels of other calcitonin gene products, results from competitive assays tended to be up to 10 times those of sandwich assays. Most laboratories use sandwich assays for measurement of calcitonin,

Table 4
Drugs affecting thyroid hormone binding

Drug/Effect	Total T4/T3	Free T4/T3
Furosemide (high-dose intravenous administration), salicylates (displaces T4, effect short lived)	Decrease	Increase by ultrafiltration Decreased in assays with marked dilution
Phenytoin, carbamazepine (decrease binding, decreased thyroid-stimulating hormone response)	Decrease	Decrease in most assays long term, may increase short term, normal by ultrafiltration
Heparin (activates lipoprotein lipase, releasing free fatty acids from triglycerides)	No effect	Increases, degree of increase rises with time of storage, accentuated with high serum triglycerides

Abbreviations: T3, triiodothyronine; T4, thyroxine.

and upper reference limits are usually up to 10 ng/L; using older assays, calcitonin upper reference limits were often 100 ng/L or higher. Even with the use of sandwich assays, calcitonin results show significant differences between methods [35], which makes it difficult to generalize information about calcitonin measurements. Assays that use monoclonal antibodies seem less likely to produce increased calcitonin levels in patients who have nonthyroid conditions than are assays using polyclonal antibodies [36].

Common causes of increased calcitonin with older assays, other than medullary thyroid carcinoma, included renal disease and acute illness. At least some of the increase in calcitonin in these states was due to the presence of other preprocalcitonin cleavage products, such as procalcitonin. Few data exist on the ability of newer calcitonin assays to produce normal results in these conditions. One recent study found that although assays using monoclonal antibodies were significantly less likely to produce elevated results, abnormal results occurred in some individuals who had either condition [36]. Other conditions, such as Hashimoto thyroiditis, may be associated with elevated calcitonin levels [37]. The use of immunometric assays can cause falsely elevated results in patients who have heterophile antibodies, as with other immunometric tests such as TSH.

The utility of calcitonin as a preoperative test in persons who have thyroid nodules (and in nonnodular thyroid disease) remains controversial. Although the German Society for Endocrinology recommends routine screening for medullary thyroid cancer using calcitonin in persons who have nodular goiter (followed by pentagastrin stimulation in patients who have elevated calcitonin) [38], others have recommended against routine calcitonin measurement [39–41].

Thyroglobulin

Thyroglobulin measurement is commonly used to evaluate eradication of thyroid tissue after treatment of differentiated thyroid carcinoma. Before thyroidectomy, the major variables that affect thyroglobulin level are thyroid mass and thyroid injury. After thyroidectomy, and particularly after elimination of remnant thyroid tissue by radioactive iodine, thyroglobulin levels are highly specific for residual thyroid carcinoma.

Thyroglobulin circulates in multiple, slightly different forms, although the variability in thyroglobulin structure is less in thyroid carcinoma than in persons who have benign disease [42]. As with other assays, the multitude of forms leads to discrepant results between different methods, and in some cases results differ by more than 40% between different assays [43]. The optimal lower limit of detection for thyroglobulin is not known; assays that detect extremely low levels (<0.1 ng/mL) detect measurable thyroglobulin in most individuals who have thyroid cancer after radioactive iodine ablation, even in the absence of residual thyroid tissue by even the most sensitive imaging techniques [44].

Commercially available kits for the measurement of thyroglobulin use the immunometric assay format. Heterophile antibodies can cause falsely increased results for thyroglobulin. The major cause for interference in thyroglobulin assays is the presence of thyroglobulin autoantibodies; an estimated 25% of patients who have thyroid cancer are antithyroglobulin positive [1]. With immunometric assays, thyroglobulin is typically falsely decreased, and results may be undetectable. Although some studies have suggested that low titer antibodies may not significantly interfere or that results are reliable if recovery (ie, measurement of thyroglobulin after addition of a known amount of thyroglobulin to samples) is close to 100% [45], other studies have shown that these approaches are unreliable for identifying clinically significant interference, especially with the expected low values in persons after thyroidectomy [3].

A single laboratory (in Dr. Carole Spencer's laboratory at University of Southern California) provides a competitive immunoassay for thyroglobulin with comparison to previous samples for true comparison of changes in results. Because interferences from heterophile antibodies are much less in competitive immunoassays and because thyroglobulin antibodies tend to produce falsely increased results with this assay format, it is often possible to detect whether significant interference is present based on comparison of results between this assay and an immunometric assay result.

Thyroid peroxidase and thyroglobulin antibodies

The methods for measuring these thyroid antibodies have changed over the years. Almost all laboratories use immune absorption assays. These are similar in approach to immunometric assays. A solid support

containing the target of the antibodies is incubated with the patient serum, and a reproducible proportion of antibody present is captured on the solid support. After washing, an antibody to human immunoglobulin, labeled in some way, is added. The amount of label is directly proportional to the amount of antibody bound. Until recently, ELISAs were the most common labels. Chemiluminescence, which has fewer interferences than enzyme assays, is becoming more commonly used. Results are often reported as units of enzyme activity present (typically reported as mU/L) or as a signal to cutoff ratio (the cutoff being the arbitrary amount of “signal” necessary to call a result positive, arbitrarily set as 1.0). Although in most cases there is good correlation between the results using immune absorption and older assay formats, which were reported as the highest dilution of serum that produces a positive result, the results are not directly equivalent. In some cases, antibodies are present in high titer but attach to antigen weakly; such samples produce low signals. With another pattern, low titer antibodies have strong antigen binding and produce high signals. High immune absorption results usually represent high titer antibodies, whereas weakly positive results are more likely to be low titer or false-positive results.

Results for antibody assays may differ with kits from different manufacturers. Part of the problem, as summarized in the NACB guidelines, is that International Reference Preparations used to try to standardize results are outdated, and these preparations are not used by all assay manufacturers [9]. It is critical in interpreting results that the reference range reported by the laboratory be used.

Thyroid peroxidase (TPO) antibodies detect the major antigen found in older thyroid microsomal antibody assays. Because TPO exists in several isoforms and degree of glycosylation, the form of TPO used can affect the ability of the test to detect antibodies in the patient’s serum and the degree to which other antibodies cross-react. Moreover, different patients develop antibodies to different forms of TPO or to different epitopes of the TPO molecule, which may affect results. This is generally more of a problem with low-avidity antibodies and with weakly positive test results. It also contributes to differences in results between laboratories [46]. In one study involving six methods, positive results with a new method correlated with positive results in the five other methods in 87% to 97% of cases [47].

There is controversy over how cutoff values for distinguishing positive and negative results for TPO antibodies should be determined. The NACB guidelines suggest using young male patients who have TSH levels between 0.5 and 2.0 mU/L. Using a “composite logarithmic gaussian distribution,” Jensen and colleagues found a cutoff of 9.8 kIU/L compared with 24 kIU/L using the NACB criteria [48].

Thyroglobulin antibodies are of most use in predicting interferences in thyroglobulin measurement to monitor differentiated thyroid cancer. Because of the large size of thyroglobulin and the presence of different forms

in the circulation, results from different assays may differ. Comparing results from different assays revealed concordance between a new method and five existing methods in 87% and 96% of samples [47].

Probably because of this, results of thyroglobulin antibody “titers” do not correlate with the degree of interference in thyroglobulin assays [49] and cannot therefore be used to “correct” thyroglobulin results for the presence of antibodies. Decreasing levels of thyroglobulin antibody, especially if they become negative after being positive, have been found to correlate with absence of residual thyroid cancer [1]; those with successful treatment lose antibodies an average of 3 years after treatment [50]. It is important for laboratories to use the same method when assessing changes in results, and changes in antibody titer should be made using frozen serum samples analyzed at the same time as new samples from the same patient [9]; however, few laboratories save samples to analyze in this fashion.

Summary

Laboratory tests of thyroid function have improved over the years, but issues remain with their performance. Standardized TSH reference limits will be difficult to establish until TSH assays agree better between laboratories. Immunometric TSH, calcitonin, and thyroglobulin assays are subject to interferences from heterophile antibodies, including rheumatoid factor, which can cause falsely high results that can lead to incorrect diagnoses. Tests of total and free thyroid hormones are subject to a variety of interferences, which are most problematic in persons with acute illness. Differences between methods remain a problem for all thyroid hormone assays.

Because of these issues, it is imperative that those who use the endocrine laboratory be familiar with the tests used for measuring thyroid hormones. Laboratories need to be familiar with the limitations of their assays and prepared to measure hormones by an alternative method in situations where interferences are possible and to interpret the results of tests performed by alternative methods. Until perfect laboratory methods are available, close cooperation between those who perform and those who use thyroid tests is necessary to assure accurate interpretation of test results and appropriate patient care.

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