Saudi Journal of Medicine and Public Health

https://saudijmph.com/index.php/pub

https://doi.org/10.64483/202522437

Laboratory Assessment of Thyroid Function: Integrated Diagnostic Pathways for Laboratory Technicians and Endocrinology Specialists

Abdullah Mohammed Almarshud [(1)] , Badour Mayof Alanazi [(2)] , Norah Saad Almutairi [(3)] , Mehad Abdulkreem Abdulhameed Alkhifi [(4)] , Marwah Ahmed Muqtiri [(5)] , Hashim Saad Aldukhayni [(6)] , Amal Ali Soliman Gahtani [(7)] , Nasser Najem Alothman [(8)] , Abdullah Al-Ghamdi [(9)] , Amin Ibrahim Ahmed Otaif [(10)] , Mohammed Aqeel Mohammed Alsharari [(2)]

(1) Qassim Health Cluster Alrabieia,Ministry of Health, Saudi Arabia,
(2) Ministry Of Health, Saudi Arabia,
(3) Maternity And Child Hospital,Ministry of Health, Saudi Arabia,
(4) Prince Mohammed Bin Abdulaziz Hospital In Riyadh,Ministry of Health, Saudi Arabia,
(5) Regional Laboratory & Central Blood Bank In Jizan,Ministry of Health, Saudi Arabia,
(6) King Khalid Hospital Alkharj,Ministry of Health, Saudi Arabia,
(7) Jazan Regional Laboratory,Ministry of Health, Saudi Arabia,
(8) Tumaer General Hospital , Ministry of Health, Saudi Arabia,
(9) King Abdullah Bisha Hospital,Ministry of Health, Saudi Arabia,
(10) Ma'taq Al-Asm Health Center,Ministry of Health, Saudi Arabia

Abstract

Background: Thyroid hormones regulate metabolism, cardiovascular function, and neuropsychiatric health. Even minor deviations in thyroid status can lead to multisystem disorders, making thyroid function tests (TFTs) essential in clinical practice.

Aim: To outline integrated diagnostic pathways for thyroid function assessment, emphasizing laboratory procedures, interpretive frameworks, and quality control for accurate diagnosis and monitoring.

Methods: A comprehensive review of thyroid physiology, etiologic categories, epidemiologic drivers, and laboratory methodologies was conducted. The article details specimen handling, immunoassay principles, interference factors, and quality management systems to ensure reliable TFT results.

Results: TSH remains the primary screening marker due to its sensitivity, but FT4 and FT3 measurements are critical in subclinical disease, central hypothyroidism, and treatment monitoring. Interfering factors—such as heterophile antibodies, binding protein abnormalities, and medications—can distort results, necessitating confirmatory strategies like equilibrium dialysis or mass spectrometry. Quality assurance through internal/external QC and patient-based monitoring minimizes analytical errors. Clinical interpretation must integrate physiologic context, comorbidities, and exposure history to avoid misdiagnosis.

Conclusion: Accurate thyroid diagnostics require a multidisciplinary approach combining physiologic insight, rigorous laboratory practice, and contextual interpretation. Understanding assay limitations and interference mechanisms is vital for preventing diagnostic errors and optimizing patient care.

Keywords: Thyroid function tests, TSH, FT4, FT3, immunoassay, assay interference, quality control, hypothyroidism, hyperthyroidism.

Introduction

The thyroid gland is a highly specialized endocrine organ located in the anterior neck, typically positioned inferior to the larynx and anterior to the upper trachea. Its bilobed, "butterfly-shaped" anatomy—two lateral lobes connected by an isthmus—reflects not only a distinctive structural configuration but also an essential physiologic role in systemic homeostasis. The gland synthesizes and secretes thyroid hormones, primarily thyroxine (T4) and triiodothyronine (T3), which exert widespread effects on nearly every organ system. Although T4 is produced in greater quantity, T3 represents the more biologically active hormone at the tissue level, and much of circulating and intracellular T3 derives from peripheral conversion of T4. The production, secretion, and feedback regulation of these hormones are governed by the hypothalamic–pituitary–thyroid (HPT) axis, a tightly controlled endocrine network that maintains circulating hormone concentrations within narrow physiologic limits. In this axis, hypothalamic thyrotropin-releasing hormone

*Corresponding author e-mail: Amalmarshud@Moh.Gov.Sa (Abdullah Mohammed Almarshud).
Receive Date: 01 December 2025, Revise Date: 28 December 2025, Accept Date: 31 December 2025

stimulates pituitary secretion of thyroid-stimulating hormone (TSH), which in turn drives thyroidal iodine uptake, hormone synthesis, and hormone release. Circulating T4 and T3 then provide negative feedback at both pituitary and hypothalamic levels, ensuring stability of thyroid hormone availability over time.[1] Thyroid hormones are central regulators of basal metabolic rate and energy utilization, influencing oxygen consumption, thermogenesis, and the metabolic handling of carbohydrates, lipids, and proteins. Their effects extend beyond metabolism to include cardiovascular performance, neuromuscular function, gastrointestinal motility, reproductive physiology, and neuropsychiatric well-being. Consequently, even modest deviations in thyroid hormone status can manifest as multisystem clinical syndromes. Hypothyroidism may present with fatigue, weight gain, cold intolerance, constipation, bradycardia, and cognitive slowing, whereas hyperthyroidism is often associated with weight loss, heat intolerance, tremor, anxiety, tachyarrhythmias, and sleep disturbance. Importantly, thyroid dysfunction may mimic or exacerbate common medical and psychiatric conditions, which explains why clinicians frequently consider thyroid evaluation during workups for nonspecific symptoms such as unexplained weight change, mood disturbance, impaired concentration, or new-onset cardiovascular abnormalities.[1]

Thyroid function tests (TFTs) comprise a set of biochemical assessments designed to evaluate the functional status of the thyroid gland by quantifying circulating markers of the HPT axis, most commonly TSH, free T4 (and sometimes total T4), and T3 (free or total, depending on the clinical context). These tests are among the most frequently ordered investigations in modern laboratory medicine because they offer high clinical utility, strong diagnostic performance, and broad applicability across outpatient and inpatient settings. From an endocrinology perspective, TFTs serve two principal roles: they establish a biochemical diagnosis of thyroid dysfunction and they support longitudinal monitoring of therapy, including levothyroxine replacement for hypothyroidism, antithyroid drugs for hyperthyroidism, and hormone suppression strategies in selected thyroid malignancies.[2] The interpretive value of TFTs rests on their physiologic coupling. TSH, secreted by the anterior pituitary, often functions as the most sensitive initial marker because it changes logarithmically in response to relatively small shifts in circulating thyroid hormone levels. In primary hypothyroidism, diminished thyroid hormone production leads to elevated TSH; in primary hyperthyroidism, excess thyroid hormone suppresses pituitary TSH secretion. However, the laboratory assessment of thyroid function is not always straightforward. Non-thyroidal illness, medications, pregnancy, pituitary or hypothalamic disease, and variations in binding proteins can alter measured hormone concentrations or disrupt expected patterns, requiring careful correlation with clinical findings and, at times, the use of additional specialized assays. For laboratory technicians, this underscores the importance of pre-analytical awareness, correct specimen handling, and familiarity with assay methodology and interferences. For endocrine specialists, it highlights the need to interpret TFTs within a broader diagnostic framework that considers patient physiology, comorbid illness, and the possibility of central (secondary) thyroid disorders.[2] Given the thyroid gland's far-reaching influence on human physiology and the high prevalence of thyroid-related complaints in clinical practice, TFTs occupy a critical position at the intersection of laboratory diagnostics and endocrine decision-making. They provide an evidence-based mechanism for identifying clinically overt disease, detecting subclinical dysfunction, guiding treatment initiation or adjustment, and preventing complications of under- or overtreatment. Moreover, they are frequently incorporated into evaluations for conditions that may be linked to thyroid hormone imbalance, including excessive weight gain, cognitive slowing, atrial fibrillation, and anxiety-related disorders, reflecting the breadth of thyroid hormone effects on metabolic, neurologic, and cardiovascular systems [1][2].

Etiology and Epidemiology

A coherent understanding of thyroid disease begins with an appreciation of normal hypothalamic–pituitary–thyroid (HPT) axis physiology, because most pathological states reflect either disruption of this regulatory loop or disease intrinsic to the thyroid gland that secondarily distorts feedback signals. In physiologic conditions, thyrotropin-releasing hormone (TRH) secreted by the hypothalamus stimulates the anterior pituitary to release thyroid-stimulating hormone (TSH). TSH then acts on the thyroid follicular cell through the TSH receptor to promote iodide uptake, thyroglobulin synthesis, iodination, coupling reactions, and release of thyroid hormones—predominantly thyroxine (T4) and smaller quantities of triiodothyronine (T3). Circulating hormone production is characteristically weighted toward T4: approximately 85% to 90% of secreted hormone is T4, while 10% to 15% is T3. In plasma, the vast majority of both T4 and T3 is protein-bound—approximately 99.5%—principally to thyroxine-binding globulin (TBG), and to a lesser extent albumin and transthyretin (pre-albumin). This binding functions as a circulating reservoir that buffers hormone fluctuations and determines the measured "total" hormone concentration, while the unbound fraction ("free" T4 and free T3) represents the biologically active pool available to tissues. Thyroid hormones enter cells via specific membrane transport proteins, and these transport mechanisms

influence both cellular uptake and, indirectly, the kinetics of hormone release and distribution [1][2][3].

A critical layer of regulation occurs beyond the thyroid gland through the activity of iodothyronine deiodinases (DIOs), which govern tissue-level thyroid hormone activation and inactivation. Type 1 deiodinase facilitates peripheral conversion of T4 to T3, supporting systemic availability of active hormone. Type 2 deiodinase converts T4 to T3 within the hypothalamus and pituitary and is central to negative feedback regulation because it shapes the local intracellular T3 signal sensed by the HPT axis. In contrast, type 3 deiodinase inactivates thyroid hormones by converting T4 to reverse T3 (rT3) and T3 to 3,5-diiodo-L-thyronine (T2), both biologically inactive products. Through these pathways, tissues can locally "tune" thyroid hormone effect independent of serum concentrations, and this helps explain why acute systemic illness may produce characteristic laboratory patterns without primary thyroid failure. Ultimately, T3 is the principal biologically active ligand that binds nuclear thyroid hormone receptors and modulates gene transcription across target tissues.[3]

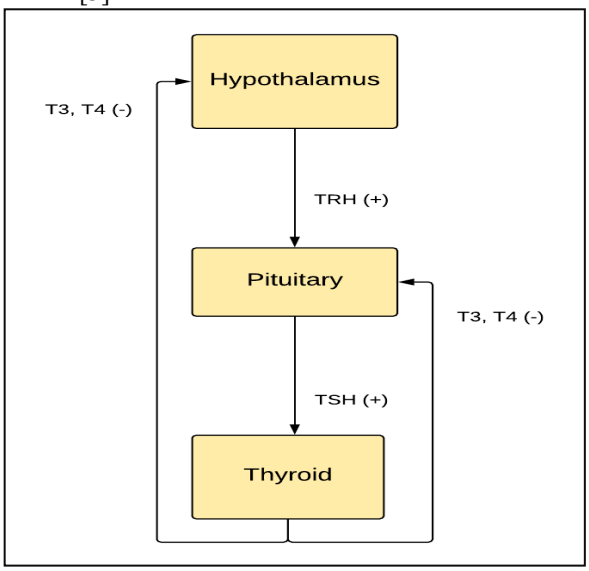

Fig. 1: The Hypothalamic-Pituitary-Thyroid Axis.

Etiologic frameworks: where thyroid dysfunction originates

Thyroid disorders arise from multiple etiologic pathways that can be organized into primary (thyroidal) disease, secondary (pituitary) or tertiary (hypothalamic) disease, and a set of special categories that blur the boundaries—such as hormone resistance states, medication effects, pregnancy-associated thyroid dysfunction, and nonthyroidal illness. In practice, the laboratory assessment of thyroid function depends on recognizing which component of the axis is driving the abnormality. Primary thyroid disease typically produces inverse changes in TSH relative to free hormone levels: in primary hypothyroidism, decreased T4/T3 production results in elevated TSH; in primary hyperthyroidism, excess hormone suppresses TSH. By contrast, central hypothyroidism due to pituitary or hypothalamic dysfunction may produce low or inappropriately normal TSH despite low free T4, and central hyperthyroidism (less common) may present with non-suppressed or elevated TSH despite elevated free T4 and/or free T3. This physiologic logic provides the conceptual scaffold for interpreting thyroid function tests in both outpatient screening and inpatient diagnostic contexts. Within primary disease, thyroiditis and autoimmune thyroid disease occupy a central role. "Thyroiditis" encompasses inflammatory disorders that may be painless or painful, transient or chronic, and autoimmune or infectious. Autoimmune thyroiditis includes a spectrum such as Hashimoto thyroiditis, atrophic thyroiditis, juvenile thyroiditis, postpartum thyroiditis, silent thyroiditis, and focal thyroiditis.[5] These entities differ in clinical timing, degree of thyroid hormone leakage from inflamed tissue, and likelihood of progression to permanent hypothyroidism, but they share an immunologically mediated injury mechanism that compromises follicular architecture. In contrast, suppurative thyroiditis is a rare infectious inflammation of the thyroid gland caused by bacteria, fungi, or parasites; although uncommon, it is clinically important because it may mimic other neck pathology and can be life-threatening if unrecognized.[5]

Autoimmune thyroid disease more broadly includes Graves disease and Hashimoto thyroiditis and may occur in association with polyglandular autoimmune syndromes type 1 and type 2.[6] Graves disease is characterized by stimulating autoantibodies against the TSH receptor, resulting in sustained hormone overproduction and diffuse gland hyperactivity. Hashimoto thyroiditis, in contrast, is typically associated with destructive autoimmune mechanisms (often with anti-thyroid peroxidase and anti-thyroglobulin antibodies), leading to progressive loss of function and hypothyroidism. These autoimmune disorders are also clinically relevant because they cluster with other immune-mediated conditions and display strong sex and age distributions, shaping epidemiologic patterns of thyroid dysfunction seen in community practice. Hypothyroidism can be classified into primary and secondary categories.[7] Primary hypothyroidism includes congenital or developmental gland dysgenesis, chronic autoimmune thyroiditis, postpartum thyroiditis, and subacute thyroiditis, as well as iatrogenic etiologies such as total or subtotal thyroidectomy and radiation-induced tissue destruction. It also includes defective hormone synthesis pathways and infiltrative diseases of the gland, including sarcoidosis, amyloidosis, scleroderma, hemochromatosis, and lymphoma. Importantly, several medications and exposures can induce hypothyroidism or disrupt thyroid function testing, including iodine-containing compounds,

radiographic contrast agents, and lithium, which can impair hormone synthesis or release. Iodine deficiency remains a major global cause of hypothyroidism and goiter, highlighting that thyroid disease epidemiology is not uniform worldwide and is influenced by nutritional environment and public health policy.

Secondary hypothyroidism arises from hypothalamic or pituitary disease, including invasive tumors or lesions, intracranial surgery, irradiation, head injury, infarction, lymphocytic hypophysitis, infiltrative disorders (sarcoid, hemochromatosis, histiocytosis X), chronic infections (tuberculosis, syphilis, mycoses), genetic defects involving the TSH beta subunit or receptor mutations, and idiopathic causes.[7] Clinically, this category is crucial because reliance on TSH alone may miss central disease, necessitating free T4 assessment and broader pituitary evaluation in the appropriate clinical context. Hyperthyroidism likewise includes primary, secondary, and "other" causes.[8] Primary hyperthyroidism is most commonly autoimmune (Graves disease) but also includes toxic multinodular goiter and toxic adenoma, in which autonomously functioning nodules produce hormone independent of TSH. Hashitoxicosis and postpartum thyroiditis may cause transient thyrotoxicosis through destructive release of preformed hormone rather than increased synthesis, a distinction that influences both laboratory patterns and treatment choice. Secondary hyperthyroidism may result from a pituitary tumor producing TSH, leading to elevated or inappropriately normal TSH in the presence of elevated thyroid hormones. Additional causes include ectopic thyroid tissue, struma ovarii, human chorionic gonadotropin (hCG)-mediated thyrotoxicosis due to hCG-secreting tumors or gestational hyperthyroidism, thyroid destruction from viral or bacterial thyroiditis, iodine-induced hyperthyroidism, and thyrotoxicosis factitia due to exogenous hormone ingestion.[8] This broad list underscores that "hyperthyroidism" is not a single disease entity but a biochemical syndrome requiring etiologic precision—particularly because some causes are treated with antithyroid drugs while others require supportive care, withdrawal of exogenous hormone, or management of the underlying tumor.

Finally, thyroid cancer has a multifactorial etiology that incorporates sex, age, prior radiation exposure, and personal or family history.[9] Although cancer is not a primary focus of routine thyroid function testing, it intersects with laboratory assessment through postoperative surveillance strategies, thyroglobulin monitoring, and the use of TSH suppression therapy in selected differentiated thyroid cancers. Beyond these classic categories, two additional frameworks are essential for laboratory and endocrine practice. The first is hormone resistance syndromes, such as genetic mutations causing TSH resistance at the receptor level, which may produce unusual laboratory patterns (for example, elevated TSH with normal or elevated thyroid hormones) that can be mistaken for assay error or nonadherence unless considered explicitly.[4] The second is nonthyroidal illness, sometimes referred to as euthyroid sick syndrome, in which acute or chronic systemic disease—including acute psychiatric illness—alters deiodinase activity, binding proteins, and hypothalamic signaling, resulting in reduced T3, variable TSH, and altered rT3 without intrinsic thyroid failure.[4] This state is epidemiologically common in hospitalized patients and is a major source of diagnostic confusion and unnecessary treatment if thyroid function tests are interpreted without clinical context [4][5][6][7].

Epidemiology: patterns, populations, and drivers of thyroid disease burden

Thyroid disorders are among the most prevalent endocrine conditions encountered worldwide, and their epidemiology reflects an interplay among autoimmunity, iodine nutrition, sex and age distributions, pregnancy-related immune shifts, medication exposure, and comorbid systemic illness. While exact incidence and prevalence vary by region and study design, several consistent epidemiologic principles guide clinical reasoning. Autoimmune thyroid disease is markedly more common in women than in men, and incidence increases with age for many phenotypes, contributing to a high burden of subclinical hypothyroidism in older adult women and a clinically significant burden of Graves disease in younger to middle-aged women. These sex differences are likely influenced by immune regulation, genetic factors, and hormonal milieu, and they matter operationally because they shape screening practices and thresholds for testing in symptomatic patients. Iodine availability is a dominant environmental determinant of thyroid disease epidemiology. In iodine-sufficient regions—often those with established salt iodization programs—the overall burden shifts toward autoimmune thyroiditis, nodular disease, and iatrogenic thyroid dysfunction related to medications and medical interventions. In iodine-deficient regions, goiter prevalence rises, hypothyroidism becomes more common, and nodular autonomy may develop over time, predisposing to toxic multinodular goiter and iodine-induced hyperthyroidism when iodine is introduced rapidly. These transitions are not merely theoretical: they explain why a patient's geographic origin, dietary patterns, and public health context can meaningfully change pretest probability for specific thyroid etiologies. Pregnancy introduces a unique epidemiologic domain because immune tolerance changes, hCG alters thyroid physiology, and postpartum immune rebound increases susceptibility to autoimmune thyroiditis. Postpartum thyroiditis is a recognized cause of transient

hyperthyroid and hypothyroid phases, and gestational thyrotoxicosis can occur through hCG-mediated stimulation, especially in hyperemesis gravidarum or hCG-secreting states.[4][8] These pregnancy-associated patterns have practical implications for laboratory interpretation because reference ranges for TSH and free hormones can shift during gestation, and because misclassification can result in overtreatment or missed risk to maternal-fetal health [4][8].

Medication exposure is another increasingly important epidemiologic driver, particularly in healthcare systems with high utilization of agents known to affect thyroid function. Lithium, iodinated contrast, iodine-containing medications, and some immunotherapies can precipitate hypothyroidism, hyperthyroidism, or thyroiditis phenotypes, and they also complicate laboratory interpretation by affecting hormone binding or conversion.[7][8] As populations age and polypharmacy increases, drug-induced thyroid dysfunction becomes more prominent in clinical practice. Additionally, expanding use of high-resolution imaging increases detection of incidental thyroid nodules and subsequent workup; while this is not strictly "thyroid function" epidemiology, it changes the volume of thyroid-related laboratory testing and downstream endocrine evaluations. Nonthyroidal illness contributes substantially to the apparent epidemiology of "abnormal thyroid tests," especially in hospitalized settings. Many acutely ill patients exhibit reduced T3 and altered TSH dynamics due to shifts in deiodinase activity, cytokine signaling, and binding protein levels.[4] This phenomenon can inflate the apparent prevalence of thyroid dysfunction if test results are not interpreted in context and may lead to inappropriate diagnosis of hypothyroidism or hyperthyroidism. From an epidemiologic perspective, it reinforces why test ordering strategy matters: indiscriminate screening in severely ill patients may identify biochemical abnormalities that represent adaptive responses rather than disease requiring treatment. Central hypothyroidism and pituitary-driven hyperthyroidism are epidemiologically less common than primary thyroid disease but are clinically high-stakes because they can be missed if clinicians rely exclusively on TSH. Populations at higher risk include those with known pituitary tumors, prior cranial irradiation, neurosurgical history, traumatic brain injury, or systemic infiltrative diseases.[7] While these conditions represent a smaller fraction of overall thyroid dysfunction, they account for a disproportionate share of diagnostic delays and misinterpretation, highlighting the importance of pairing laboratory strategy with etiologic reasoning. Thyroid cancer epidemiology is likewise shaped by radiation exposure, family history, and demographic factors, and although not all cancers alter thyroid hormone levels, the increasing detection of differentiated thyroid cancers in many regions has increased the population undergoing thyroidectomy and subsequent long-term hormone replacement or suppression therapy.[9] This creates an expanding cohort requiring serial laboratory monitoring, and thus, thyroid cancer indirectly contributes to the epidemiology of thyroid function testing utilization [4][7][8][9].

Etiology–epidemiology linkage in laboratory practice

From the perspective of laboratory technicians and endocrine specialists, the most important practical insight is that etiologic heterogeneity and epidemiologic context jointly determine the correct test strategy and interpretation. In populations with high autoimmune burden, TSH with reflex free T4 (and antibody testing when indicated) will capture the majority of clinically relevant dysfunction, while in hospitalized patients or those on interfering medications, test selection and timing must be more deliberate to avoid misclassification. Understanding that T3/T4 are overwhelmingly protein-bound and that binding proteins vary with pregnancy, estrogen therapy, liver disease, and systemic illness also clarifies why "total" hormone tests may diverge from free hormone measurements, and why assay methodology matters in borderline cases. Similarly, awareness of deiodinase physiology clarifies why low T3 states commonly accompany critical illness and why rT3 may rise in nonthyroidal illness, helping clinicians resist overdiagnosis in contexts where the thyroid gland itself is not failing.[3][4] In sum, thyroid disease etiology spans autoimmune inflammation, destructive and infectious thyroiditis, nodular autonomy, congenital and iatrogenic gland loss, central hypothalamic–pituitary pathology, pregnancy-related shifts, medication effects, hormone resistance syndromes, nonthyroidal illness, and multifactorial cancer risks.[4][5][6][7][8][9] Epidemiologically, the distribution of these causes is shaped by sex and age patterns, iodine nutrition, pregnancy biology, drug exposure, and the prevalence of systemic illness. Mastery of these etiologic categories and epidemiologic drivers is not academic ornamentation; it is the foundation for accurate test ordering, correct interpretation of abnormal patterns, and the avoidance of both missed diagnoses and unnecessary treatment in patients whose laboratory abnormalities reflect physiology rather than pathology.

Specimen Requirements and Procedure

Accurate measurement of thyroid hormones and pituitary signaling markers depends not only on assay selection but also on rigorous attention to specimen collection, processing, storage, and documentation. For laboratory technicians, these pre-analytical variables are often the most controllable determinants of result reliability, and they become especially consequential when clinicians are managing borderline abnormalities, monitoring therapy adjustments, or evaluating discordant patterns

(for example, abnormal TSH with apparently normal free hormone levels). Because thyroid testing is frequently used for both diagnosis and longitudinal follow-up, consistent adherence to standardized specimen requirements reduces variability and improves clinical interpretability across serial measurements. For free triiodothyronine (FT3) and free thyroxine (FT4), serum is the preferred sample matrix, and the specimen should be separated from the clot promptly after collection. Timely separation is important because prolonged contact between serum and cellular components can alter analyte stability and introduces avoidable variability, particularly when specimens are transported between sites or collected in high-volume outpatient settings. Whole blood specimens used for thyroid hormone testing remain stable at 15 ºC to 35 ºC for up to 24 hours, which is operationally helpful for facilities with delayed centrifugation workflows or transport to centralized laboratories. Once separated, serum is stable at room temperature for approximately one week, but best practice is to refrigerate samples at 4 ºC if analysis will be delayed beyond 24 hours, especially when maintaining comparability across repeated measurements.[10][11] If analysis is not performed within six days, serum should be frozen at −20 ºC or colder to preserve analyte integrity and reduce degradation-related drift.[10][11] In handling stored samples, repeated freeze–thaw cycles should be avoided because they may change protein binding dynamics and potentially degrade proteins or alter assay performance. Similarly, excessive agitation, including vigorous vortexing, can denature proteins and compromise matrix integrity, which is particularly relevant in immunoassay-based platforms that rely on predictable protein interactions.[10][11] These precautions support analytical accuracy and reduce the likelihood of unexplained variation that could be misinterpreted as a clinically meaningful change.

For thyroid-stimulating hormone (TSH), testing can be performed using either serum or plasma. While plasma samples may yield slightly higher values than serum, such differences are typically clinically insignificant for most interpretive decisions, particularly when results are clearly within or outside reference intervals.[12] Tube selection can also influence laboratory workflow and downstream processing. Importantly, no significant differences have been observed between specimens collected in serum separator tubes (SST) containing gel and those collected in tubes without gel for TSH measurement, suggesting flexibility in collection protocols as long as the laboratory maintains consistent internal validation and quality control procedures.[12] Nonetheless, laboratories should align tube selection with their validated assay requirements and institutional policies and ensure that specimen type is recorded correctly in the laboratory information system to avoid pre-analytical misclassification. Beyond specimen handling, biological variability—especially circadian rhythm—must be recognized because it affects how results are interpreted clinically, even when analytical precision is excellent. TSH displays a well-described diurnal variation, with peak levels occurring at night, typically around midnight, and lowest levels in the morning. This rhythm is clinically relevant because a borderline elevated TSH measured late in the day may not be identical to a morning measurement, particularly when values sit near decision thresholds used for defining subclinical hypothyroidism or adjusting levothyroxine dose. Moreover, circadian regulation of TSH can be disrupted during illness, which helps explain why hospitalized or acutely unwell patients may show atypical TSH patterns that do not reflect stable thyroid gland dysfunction. In central hypothyroidism, the normal nocturnal rise in TSH is characteristically absent, reflecting impaired hypothalamic or pituitary regulation rather than primary thyroid failure.[13][14] Sleep deprivation also tends to increase TSH levels, which has practical implications for patients who work night shifts, experience insomnia, or undergo overnight wakefulness prior to testing; documenting sleep disturbance can therefore assist clinicians in interpreting borderline results.[13][14]

Although TSH secretion exhibits circadian variation, it remains relatively stable across typical daytime hours in many ambulatory individuals, and reference intervals are derived from samples collected across these common clinical timeframes. Consequently, while standardization to a consistent collection time can be helpful for longitudinal monitoring—particularly when titrating hormone replacement therapy—clinically meaningful information can still be obtained from specimens drawn at various times during the day in outpatient settings.[15] The key is consistency when trending values and careful contextualization when interpreting isolated borderline results. Laboratories can support this by ensuring accurate time-of-collection recording, because clinicians may use collection timing as a clue when evaluating unexpected changes. Finally, patient characteristics such as age and gender generally do not substantially alter reference intervals for thyroid testing, though exceptions exist at the extremes of age, where physiologic set points may differ and where reference interval selection becomes more nuanced.[16] For the laboratory, this reinforces the importance of applying appropriate reference ranges provided by the assay manufacturer and validated locally, while also ensuring that clinicians have access to interpretive notes when testing is performed in populations where reference interval choice may meaningfully affect categorization. In sum, the reliability of thyroid function testing depends on disciplined specimen

processing—prompt serum separation, appropriate storage temperatures, avoidance of repeated freeze–thaw cycles and excessive agitation—and an informed awareness of physiologic variation such as circadian TSH rhythm and its disruption in illness.[10][11][12][13][14][15][16]

Diagnostic Tests

Biochemical testing forms the cornerstone of modern thyroid diagnostics because the clinical manifestations of thyroid dysfunction are often nonspecific, overlap with common cardiometabolic and neuropsychiatric conditions, and vary substantially by age, comorbidity burden, pregnancy status, and the acuity of illness. In practice, laboratory confirmation is not merely supportive; it is decisive for distinguishing true endocrine pathology from mimicking syndromes, for defining disease severity (overt versus subclinical), and for monitoring response to therapies that require tight titration to avoid long-term harm. The diagnostic test strategy therefore rests on understanding the physiologic architecture of the hypothalamic–pituitary–thyroid axis and on recognizing contexts in which standard marker relationships are altered. The foundational diagnostic tests used to assess thyroid function include serum thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). These analytes provide a linked assessment of pituitary signaling (TSH) and thyroid hormone output (T3/T4). For routine care, the most informative measures are generally TSH combined with free hormone measurements—free T4 (FT4) and, when indicated, free T3 (FT3)—because these "free" fractions represent biologically active hormone not bound to circulating transport proteins. Beyond this core panel, several adjunct biochemical tests help refine etiology, confirm autoimmunity, evaluate nodular disease, and support oncology-related decision-making. These include thyroglobulin (Tg), thyroglobulin antibody (Tg-Ab), anti-thyroid peroxidase antibody (aTPOAb), thyrotropin receptor antibody (TRAb), and calcitonin.[17] Each of these tests occupies a specific diagnostic niche: autoantibodies clarify autoimmune mechanisms, Tg and Tg-Ab are central in differentiated thyroid cancer follow-up and sometimes in evaluating destructive thyroiditis patterns, and calcitonin supports assessment when medullary thyroid carcinoma is a concern [17].

TSH remains the primary gateway test in most diagnostic algorithms. Contemporary third-generation immunoassays provide high analytical sensitivity and allow clinicians to detect subtle changes that precede overt alterations in circulating thyroid hormones. This performance profile explains why TSH is widely recommended as the initial screening test when thyroid dysfunction is suspected.[17] In classic primary thyroid disease, the directionality of TSH offers immediate interpretive value: elevated TSH typically suggests hypothyroidism, reflecting reduced thyroid hormone production and loss of negative feedback, whereas suppressed TSH usually indicates hyperthyroidism due to excess circulating hormone. However, the clinical utility of TSH is not absolute. There are important scenarios in which exclusive reliance on TSH can be misleading, such that simultaneous measurement of FT4 and, in selected situations, FT3 becomes necessary to avoid diagnostic error.[17] Subclinical thyroid disease illustrates one such scenario. In subclinical hypothyroidism, TSH may be elevated while FT4 remains within reference range, and the clinical decision-making hinges on TSH magnitude, symptom burden, pregnancy considerations, cardiovascular risk, and antibody status. Similarly, subclinical hyperthyroidism may present with suppressed TSH and normal FT4/FT3, a pattern that is clinically meaningful because it can increase risk for atrial fibrillation and bone loss despite the absence of overt biochemical thyrotoxicosis. In these subclinical states, TSH is sensitive, but it does not fully describe hormone availability at the tissue level, and free hormone measurements help confirm the classification and guide management thresholds. Hospitalized patients and those with acute systemic illness represent another high-risk context for misinterpretation. Nonthyroidal illness can disturb TSH secretion dynamics and peripheral hormone metabolism, producing patterns that mimic hypothyroidism or hyperthyroidism without intrinsic thyroid pathology. Additionally, central (secondary) hypothyroidism due to pituitary or hypothalamic disease may present with low or "inappropriately normal" TSH despite low FT4, making TSH alone an unreliable screen for central disease. For these reasons, when evaluating hospitalized patients, patients with suspected pituitary disease, or those with unexplained symptoms inconsistent with the TSH result, clinicians often require FT4 (and sometimes FT3) to establish whether the hormonal output itself is abnormal.[17]

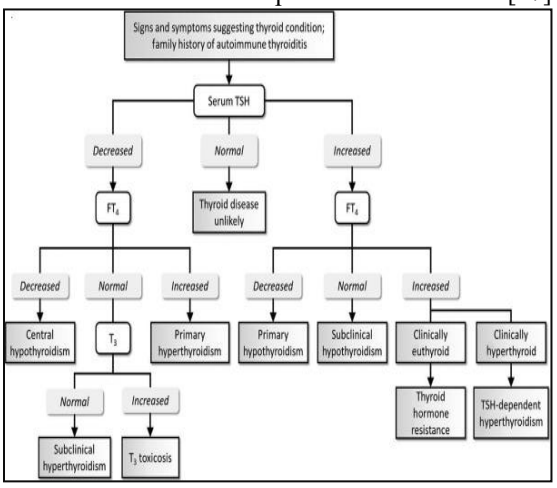

Fig. 2: Laboratory Assessment of Thyroid functions.

Therapeutic transitions are a further setting in which TSH alone may mislead. During the early phases of treatment for hyperthyroidism or

hypothyroidism, TSH can lag behind changes in circulating hormone levels because pituitary adaptation is slower than peripheral hormone shifts. For example, after initiating antithyroid therapy, FT4 and FT3 may normalize before TSH recovers from suppression; conversely, after starting levothyroxine for hypothyroidism, FT4 may rise into the desired range before TSH equilibrates. In these situations, free hormone measurements provide a more immediate reflection of biochemical response and prevent inappropriate dose adjustments based solely on a delayed TSH response.[17] Measurement of FT3 and FT4 is generally preferred over total T3 and total T4 in many clinical contexts because free hormone concentrations are less influenced by fluctuations in thyroxine-binding globulin (TBG). Altered TBG levels can occur during pregnancy, acute illness, and with several medications that modify binding protein synthesis or binding dynamics, including estrogen, tamoxifen, opiates, androgens, and glucocorticoids.[17] In such settings, total hormone concentrations may shift upward or downward without a true change in tissue-available hormone, potentially creating apparent thyroid dysfunction where none exists. Free hormone testing therefore improves diagnostic specificity when binding protein abnormalities are likely. At the same time, laboratories and clinicians must recognize that FT3 and FT4 exist at picomolar concentrations in blood, which makes their measurement analytically more challenging and more susceptible to assay limitations or interferences than many routine chemistry analytes.[17] This reality has practical implications: discordant results (for example, a clinically euthyroid patient with unexpectedly abnormal FT4) may require repeat testing, evaluation for assay interference, review of medications and supplements (including high-dose biotin), and careful clinical correlation rather than immediate diagnostic labeling. In summary, the diagnostic approach to thyroid dysfunction begins with highly sensitive TSH testing and is strengthened by concurrent FT4 and FT3 assessment when clinical context warrants.[17] Etiologic clarification and risk stratification frequently depend on adjunct markers such as Tg, Tg-Ab, aTPOAb, TRAb, and calcitonin, each providing targeted information beyond simple functional status.[17] For both laboratory professionals and endocrine clinicians, high-quality thyroid diagnostics require a disciplined pairing of assay selection with clinical context—recognizing when TSH is sufficient for screening and when free hormone and antibody testing are essential to avoid misinterpretation and to support precise, patient-centered management.

Testing Procedures

Thyroid function testing relies on immunochemical, and, in selected circumstances, physicochemical measurement techniques designed to quantify pituitary signaling (TSH) and circulating thyroid hormones (T4 and T3) in either free or total form. Because thyroid analytes span markedly different concentration ranges and exist in complex equilibrium with binding proteins, no single method is ideal for every clinical scenario. Instead, laboratories choose platforms that balance analytical performance, throughput, cost, and clinical need, while clinicians interpret results with an awareness of method-specific limitations. Understanding the core testing procedures is therefore essential for both laboratory technologists, who must ensure analytical integrity and quality control, and endocrine specialists, who must recognize when apparent biochemical patterns may reflect assay artifacts rather than true physiology. TSH is most commonly measured using automated chemiluminescent immunoassays (CLIA). In a typical “sandwich” format, chemiluminescent-labeled antibodies bind to TSH in the patient’s specimen, and the resulting immune complex is then captured by a second antibody immobilized on a solid phase. Following separation of unbound components, the chemiluminescent signal is generated and quantified; the emitted light is proportional to the amount of TSH present in the sample. This approach has supported the evolution from early low-sensitivity assays to modern third-generation assays that can measure very low TSH concentrations with clinically meaningful precision. Third-generation TSH assays are characterized by a coefficient of variation below 20% at very low concentrations (on the order of milli–international units per liter), enabling more reliable distinction among suppressed TSH states and improving diagnostic discrimination for hyperthyroid conditions.[18] By contrast, first-generation assays had limited functional sensitivity and could largely separate only overt hypothyroid from euthyroid states. Second-generation assays extended analytical sensitivity into subnormal ranges and could detect low TSH values, but they still lacked sufficient precision at the lowest levels to consistently differentiate primary hyperthyroidism from other etiologies of low TSH, including nonthyroidal illness, medication effects, or early treatment response.[18] For clinical care, this generational improvement matters because the degree of TSH suppression can influence decision-making in thyrotoxicosis, subclinical hyperthyroidism, and thyroid cancer follow-up where TSH targets may be intentionally modified [18].

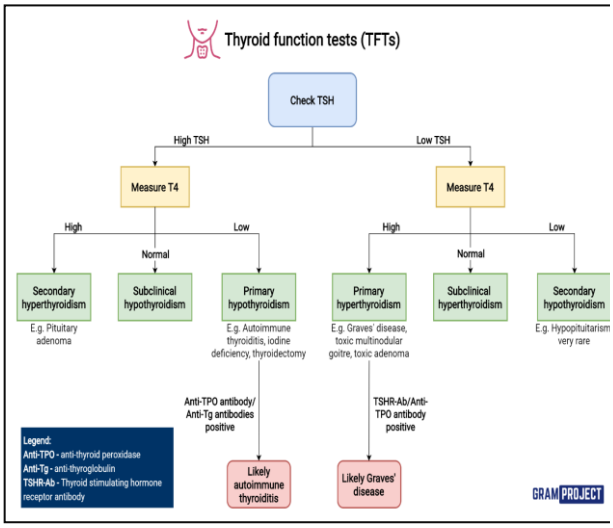

Fig. 3: Thyroid function tests.

In contrast to TSH, measurement of free thyroid hormones is more analytically challenging because FT4 and FT3 exist at picomolar concentrations and in dynamic equilibrium with protein-bound pools. Methodologically, free hormone assays may be described as direct or indirect. Direct methods physically separate free hormone from protein-bound hormone before quantification, thereby aligning closely with the physiologic definition of "free." Two well-established direct approaches are equilibrium dialysis and ultrafiltration. Equilibrium dialysis allows free hormone to diffuse across a semi-permeable membrane until equilibrium is reached, after which the free fraction is measured. Ultrafiltration uses size-selective membranes to separate free hormone from larger protein-bound complexes. These methods provide strong analytical validity and are often used as reference procedures, particularly when results are discordant with clinical findings or when binding protein abnormalities make immunoassay estimates unreliable.[19] Indirect methods, by contrast, estimate free hormone concentration without physically separating free from bound fractions. Most high-throughput FT4 and FT3 tests used in routine laboratories are immunoassays that infer free hormone levels based on binding interactions within the assay system. These immunoassays are commonly categorized as 1-step or 2-step methods. In one-step methods, specimen and reagents are incubated together without an intermediate wash; in two-step methods, a wash step removes unbound serum components before introducing a tracer, reducing the influence of binding proteins and some interferences. Although widely used because of speed and automation, indirect immunoassays can be vulnerable to alterations in binding proteins, severe illness effects, and certain assay interferences, which is why direct measurement may be preferred in complex or high-stakes cases.[19]

Total T4 and total T3 measurements differ from free hormone testing in two important ways: first, the analytes are present at nanomolar concentrations, making them easier to detect analytically; second, total hormone reflects both bound and free pools, which can be advantageous when used thoughtfully but misleading when binding proteins are altered. Total hormone testing may still be clinically useful to corroborate free hormone results or to interpret thyroid status when free hormone assays are suspected to be inaccurate. From a methodological standpoint, mass spectrometry is considered the gold standard for measurement of thyroid hormones because it provides high specificity and robust quantification across a range of concentrations and matrices. Nevertheless, many laboratories continue to use competitive immunoassays for total T3 and T4, often employing displacing agents that release hormone from serum binding sites so it can compete for antibody binding within the assay. While these immunoassays are practical and scalable, they have shown poor agreement across platforms and can correlate imperfectly with mass spectrometry, reflecting differences in antibodies, calibrators, matrix effects, and interference susceptibility.[20][21][22] For clinicians, this variability means that serial monitoring should ideally be performed using the same laboratory method when possible, and that unexpected changes should prompt consideration of methodological differences rather than immediate conclusions about physiology. Overall, thyroid testing procedures illustrate a broader principle in laboratory medicine: analytical method shapes clinical interpretation. High-sensitivity chemiluminescent immunoassays have made TSH an exceptionally powerful screening and monitoring tool,[18] whereas the inherent complexity of free hormone measurement requires careful selection between direct reference approaches and widely available immunoassays depending on context.[19] Total hormone assays remain accessible and useful, but their immunoassay-based variability compared with mass spectrometry warrants cautious interpretation, particularly when results are discordant with the clinical picture or when binding protein states are altered.[20][21][22]

Interfering Factors

Accurate interpretation of thyroid function tests requires recognition that many commonly used assays—particularly automated immunoassays for TSH, FT4, FT3, and thyroglobulin—are vulnerable to analytical interference. These interferences can generate results that appear internally inconsistent (for example, discordant TSH and FT4 patterns), fluctuate unexpectedly between visits without a physiologic explanation, or conflict with the patient's clinical presentation. Because thyroid testing often guides long-term therapy decisions and may trigger invasive investigations, understanding the sources

and mechanisms of interference is essential for both laboratory professionals and clinicians. In practice, the goal is not simply to acknowledge that interferences exist, but to identify circumstances in which interference is plausible and to apply confirmatory strategies that prevent misdiagnosis or inappropriate treatment escalation. One of the most important categories of interference involves anti-animal antibodies, commonly referred to as heterophile antibodies. These antibodies may be present in a patient's specimen due to prior exposure to animals, therapeutic monoclonal antibodies, immunizations, occupational exposures, or idiopathic immune reactivity. In sandwich-format immunoassays, heterophile antibodies can bind nonspecifically to assay antibodies, bridging capture and detection antibodies in a way that mimics analyte binding and produces spuriously elevated results. Alternatively, they may block binding sites, preventing formation of the immune complex and causing falsely low results. Because the direction of error depends on the assay design and the specific antibody interactions, heterophile interference can present as either false-positive or false-negative findings.[23][24] Importantly, susceptibility is not limited to one analyte: TSH, FT4, FT3, and thyroglobulin (Tg) are all known to be affected by heterophile or related anti-animal antibody interference, making this a particularly relevant issue in thyroid diagnostics and thyroid cancer surveillance.[25][26][27] Clinically, suspicion should rise when the result pattern is biologically implausible—such as markedly abnormal TSH with normal free hormones, abrupt shifts that do not align with dose changes, or Tg values that do not match imaging findings or expected postoperative trends. Laboratory approaches to address this include repeating the test on a different platform, performing serial dilution studies to assess linearity, using heterophile-blocking reagents, or confirming hormone levels with reference methods such as equilibrium dialysis combined with mass spectrometry in complex cases [24][25][26][27].

A second major interference mechanism relates to disturbances in hormone-binding proteins. Free hormone immunoassays attempt to estimate the unbound fraction of T4 and T3, but because most circulating hormone is protein-bound, any alteration in binding protein concentration or binding affinity can distort the measured relationship between free and total hormone, particularly in indirect immunoassays. This includes inherited and acquired changes in binding proteins. A classic example is familial dysalbuminemic hyperthyroxinemia (FDH), a genetic condition in which albumin has altered affinity for T4, producing an abnormal free-to-total hormone relationship and potentially misleading laboratory profiles if the assay cannot adequately account for the altered binding dynamics.[28] In such settings, total T4 may be elevated while the patient remains clinically euthyroid, and immunoassay-derived free T4 may be variably affected depending on platform design. Confirmatory testing with direct free hormone measurement (equilibrium dialysis or ultrafiltration) and/or mass spectrometry for total hormone can clarify the true physiologic state. Binding equilibrium can also be perturbed by exogenous or endogenous substances that displace thyroid hormones from their binding proteins during or after specimen collection. Certain medications, increased concentrations of non-esterified fatty acids, and heparin are notable contributors. Heparin, for example, can increase circulating free fatty acids through activation of lipoprotein lipase, and these fatty acids can displace thyroid hormones from binding proteins in vitro, leading to artificially elevated measured free hormone levels even when the patient is clinically stable.[29][30] This phenomenon is particularly relevant in hospitalized patients receiving heparin or in samples collected soon after heparin administration. Similarly, drug-related displacement or altered protein binding can create misleading free hormone estimates, emphasizing the value of documenting medication exposure and timing relative to blood draw [28][29][30].

Autoantibodies represent another interfering factor that can affect both free and total hormone measurements. Antibodies directed against T3, T4, or assay components may bind hormone or interfere with antibody-analyte interactions, producing spurious elevations or reductions depending on assay configuration. This form of interference may involve FT3 and FT4 assays as well as total T3 and total T4 measurements, and it can be particularly challenging because results may vary between platforms and may change over time as antibody titers fluctuate.[31] In patients with known autoimmune disease, unexplained discordance among thyroid tests should prompt consideration of autoantibody interference and may warrant repeat testing using alternative methodologies. Finally, specimen quality can contribute to analytical bias. Although gross hemolysis or significant lipemia is generally discouraged for thyroid testing due to potential optical and matrix effects, many modern assay systems tolerate moderate hemolysis or lipemia without major interference for most analytes.[32] Nevertheless, sample integrity should not be dismissed: severe lipemia can disrupt assay signal detection, and hemolysis can introduce matrix changes that affect certain platforms. Therefore, the laboratory should maintain clear rejection criteria for grossly compromised specimens while also providing interpretive support when minor hemolysis or lipemia is present and results are clinically unexpected. In summary, thyroid immunoassays are susceptible to heterophile antibodies, binding protein disturbances

(including FDH), displacement effects from drugs, fatty acids, and heparin, and autoantibody interference—each capable of producing false-positive or false-negative thyroid results.[23][24][25][26][27][28][29][30][31] While moderate hemolysis or lipemia typically has limited effect on many methods, specimen quality remains an important pre-analytical consideration.[32] Recognizing these interfering factors—and responding with method comparison, dilution checks, blocking strategies, or reference measurement—protects patients from misdiagnosis and ensures that biochemical data remain a trustworthy foundation for endocrine decision-making.

Results, Reporting, and Critical Findings

In routine clinical practice, interpretation of thyroid function is often efficient and clinically decisive because the combination of TSH and free thyroxine (FT4) typically classifies patients into euthyroid, hypothyroid, or hyperthyroid categories with high accuracy.[33] In primary thyroid disease, the expected biochemical patterns are familiar: elevated TSH with low FT4 suggests primary hypothyroidism, while suppressed TSH with elevated FT4 indicates primary hyperthyroidism. A normal TSH, in most ambulatory patients, is generally sufficient to exclude clinically significant primary thyroid dysfunction, particularly when symptoms are nonspecific and the pretest probability for thyroid disease is low.[36] As a result, laboratories commonly report TSH as the initial or reflex test, with FT4 (and sometimes FT3) added when the TSH is abnormal or when the clinical context suggests that TSH may be unreliable. Despite this typical clarity, thyroid results can occasionally be atypical or inconsistent with the patient's presentation. Such discordance can arise from several domains: medication effects, physiologic states (notably pregnancy), rare acquired or genetic hypothalamic–pituitary disorders (including TSH-secreting pituitary adenomas, or TSHomas, and thyroid hormone resistance, THR), and concurrent systemic illness that alters thyroid hormone metabolism or pituitary signaling.[3][34] For this reason, high-quality reporting requires not only numerical result release but also disciplined interpretive awareness—especially when a laboratory pattern is internally inconsistent (for example, normal TSH with high FT4) or when results shift abruptly without a plausible clinical explanation.

A central interpretive concept is that TSH responds to circulating FT4 in a log–linear manner: relatively small changes in FT4 can produce disproportionately large changes in TSH.[35] This relationship explains why TSH is a sensitive early marker in many primary thyroid disorders. In developing hypothyroidism, TSH may rise before FT4 falls below the reference interval, producing a "subclinical" presentation characterized by abnormal TSH with normal FT4. Conversely, in early hyperthyroidism, TSH may become suppressed before FT4 becomes clearly elevated. Consequently, when laboratories and clinicians engage in serial monitoring—such as adjusting levothyroxine dosing or titrating antithyroid therapy—TSH trends should be interpreted with this log–linear physiology in mind, and small FT4 changes should not be dismissed simply because they remain within the reference interval.[35] However, there are clinically important scenarios in which TSH is not a reliable standalone indicator of thyroid status. TSH may remain suppressed for a prolonged period after treatment of thyrotoxicosis, even when FT4 normalizes, because pituitary recovery lags behind peripheral hormone correction.[36] TSH may also be transiently lowered by medications such as glucocorticoids, and it can be distorted in non-thyroidal illness due to altered hypothalamic signaling and peripheral deiodination changes.[36] More critically, TSH may be misleading in central (secondary) hypothyroidism, where pituitary or hypothalamic dysfunction prevents an appropriate TSH rise despite low FT4. Rare endocrine disorders such as THR or TSHoma can produce paradoxical patterns—such as non-suppressed TSH in the presence of elevated thyroid hormones—requiring careful escalation to additional testing and, often, specialist evaluation.[3][36]

Additional tests and when they are needed

When the initial TSH–FT4 pattern does not fully explain the clinical picture, additional assays can sharpen diagnosis and prognosis. Measurement of free triiodothyronine (FT3) is particularly useful when TSH is suppressed but FT4 remains normal, because this pattern can represent T3 toxicosis—an early or selective hyperthyroid state in which T3 is elevated while FT4 has not yet risen.[3][37] FT3 can also support interpretation in patients receiving T4 replacement who demonstrate high FT4 with a normal TSH, a scenario that may reflect altered deiodination dynamics rather than true hyperthyroidism. When subclinical hypothyroidism is identified, antithyroid antibody testing—most commonly anti-thyroid peroxidase antibodies—can inform prognosis because antibody positivity increases the likelihood of progression to overt hypothyroidism over time. In suspected Graves disease, thyrotropin receptor antibody (TRAb) testing is highly sensitive and specific and can confirm autoimmune hyperthyroidism when clinical signs and biochemistry are compatible.[3][37] Effective reporting in these contexts often benefits from laboratory reflex pathways or interpretive comments that prompt clinicians toward the appropriate confirmatory tests rather than repeated, unstructured retesting.

Discordant TSH and FT4 results

One of the most clinically challenging patterns is high FT4 with a normal (or non-suppressed) TSH. In many patients on levothyroxine

(T4) therapy, this can occur due to reduced peripheral deiodination or timing-related effects (for example, blood drawn soon after a dose), resulting in elevated measured FT4 without corresponding pituitary suppression.[3][37] The same general biochemical pattern may also appear in binding protein abnormalities such as familial dysalbuminemic hyperthyroxinemia (FDH), where assay behavior and altered binding can produce apparent FT4 elevation despite euthyroidism. More concerning etiologies include THR and TSHoma, both of which can produce inappropriately normal or elevated TSH in the setting of elevated thyroid hormones and require endocrine-directed evaluation beyond routine laboratory testing.[3][37] In such cases, laboratories add value by verifying results (including repeat measurement on an alternative platform if interference is suspected), ensuring appropriate flagging, and encouraging clinical correlation rather than implying a straightforward diagnosis from a single discordant data point [36][37].

Critical results and thyroid emergencies

Thyroid emergencies are uncommon but high-stakes, and laboratories play a crucial role in rapid reporting and escalation pathways. Thyrotoxic storm and myxedema coma represent life-threatening extremes of thyroid dysfunction that demand immediate clinical action. Importantly, laboratory findings alone do not confirm these diagnoses; they must be interpreted alongside the patient's presentation because severity is defined by systemic decompensation rather than hormone levels in isolation.[38] Laboratories should therefore treat markedly abnormal thyroid results as potentially urgent signals—particularly when accompanied by compatible clinical information—while acknowledging that critical illness, assay interference, and treatment effects can sometimes distort numerical values. Myxedema coma is a severe decompensated hypothyroid state in which patients may present with hypothermia, altered mental status, cardiovascular compromise, and metabolic derangements. A diagnostic scoring system can support clinical decision-making by integrating body temperature, central nervous system effects, gastrointestinal symptoms, precipitating events, cardiovascular dysfunction, and metabolic disturbances. Laboratory data—typically low or undetectable FT4 with variable TSH (high in primary hypothyroidism but sometimes low or normal in central disease)—contribute to this assessment but do not substitute for it.[39] Conversely, thyrotoxic storm is characterized biochemically by markedly elevated T4 and T3 with suppressed TSH, but diagnosis likewise relies on a scoring system that weighs fever, neurologic dysfunction, gastrointestinal manifestations, and cardiovascular instability, because the degree of biochemical elevation does not reliably predict storm severity.[38] In both emergencies, timely reporting, clear flagging of extreme results, and rapid clinician notification processes can meaningfully influence outcomes, provided that results are framed as supportive evidence within a broader clinical diagnosis rather than definitive proof. In summary, most thyroid function interpretations are straightforward using TSH and FT4 alone,[33] yet a subset of cases demands careful reporting and escalation due to physiologic variation, medication effects, systemic illness, and rare pituitary or hormone-resistance disorders.[3][34][36] Understanding the log–linear TSH–FT4 relationship improves serial monitoring,[35] while targeted add-on testing—FT3, antibody assays, and TRAb—enhances etiologic precision and prognostication.[3][37] Finally, thyroid emergencies require urgent attention and integrated clinical interpretation, with laboratories supporting rapid recognition while acknowledging that biochemical data must be interpreted within validated clinical scoring frameworks for myxedema coma and thyrotoxic storm.[38][39]

Clinical Significance

Thyroid disorders are clinically important not only because they are common, but because they frequently present with symptoms and signs that are nonspecific and overlap with a wide range of cardiometabolic, neuropsychiatric, reproductive, and general medical conditions. Fatigue, weight change, mood disturbance, palpitations, constipation, tremor, menstrual irregularity, and cognitive slowing can reflect thyroid dysfunction, yet each is also prevalent in patients without endocrine disease. For this reason, biochemical confirmation is essential to avoid both underdiagnosis and overdiagnosis. Thyroid-stimulating hormone (TSH) is widely used as the primary screening test because it is sensitive to small shifts in circulating thyroid hormones through negative feedback mechanisms. Nevertheless, the clinical significance of thyroid testing lies equally in understanding the limitations of TSH and the circumstances in which additional assays—particularly free thyroxine (FT4) and sometimes free triiodothyronine (FT3)—are required for accurate classification and safe management.[17] The limitations of TSH become particularly apparent in central hypothyroidism, where hypothalamic or pituitary dysfunction prevents an appropriate TSH rise despite low thyroid hormone levels. In such cases, reliance on TSH alone can falsely reassure clinicians and delay diagnosis, exposing patients to progressive bradycardia, dyslipidemia, cognitive impairment, and, in severe cases, life-threatening decompensation. Similarly, in hospitalized patients with acute systemic illness, TSH and peripheral thyroid hormone metabolism may be altered by non-thyroidal illness effects, making isolated results difficult to interpret without clinical context. Treatment monitoring is another setting where TSH

may mislead; after initiating therapy for hyperthyroidism, TSH can remain suppressed for weeks to months despite normalization of FT4/FT3, and after starting levothyroxine for hypothyroidism, TSH may lag behind changes in FT4. Certain medications—such as glucocorticoids, dopamine agonists, amiodarone, lithium, and iodine-containing agents—can also alter pituitary signaling, thyroid hormone synthesis, or peripheral conversion, creating patterns that are not straightforward unless the biochemical profile is interpreted with exposure history and timing in mind.[17]

Subclinical thyroid disease

Subclinical thyroid disease describes individuals who have normal circulating thyroid hormone levels but abnormal TSH. When TSH is low with normal FT4 and FT3, the condition is termed subclinical hyperthyroidism; when TSH is elevated with normal FT4, it is termed subclinical hypothyroidism. Although many patients are asymptomatic, these states carry clinically meaningful risks depending on the degree of TSH abnormality, age, comorbidities, and duration. Treatment consideration for subclinical hyperthyroidism is particularly relevant in geriatric patients and in individuals with atrial fibrillation or osteoporosis, where even mild thyroid hormone excess can accelerate bone loss and increase arrhythmia risk.[17] In subclinical hypothyroidism, treatment decisions often hinge on the TSH level and markers of autoimmune risk. A TSH greater than 10 mIU/L is commonly viewed as a threshold where the likelihood of progression and symptom burden increases, and treatment is more strongly considered. Elevated anti–thyroid peroxidase antibody (aTPOAb) levels also support a higher risk of progression to overt hypothyroidism, making therapy or closer monitoring clinically justified. Comorbidities such as hypercholesterolemia and infertility can further tilt decision-making toward treatment, because thyroid optimization may improve lipid profiles and reproductive outcomes in selected patients.[17]

Hyperthyroidism

Hyperthyroidism most commonly results from increased hormone production within the thyroid gland and is biochemically characterized by elevated T4 and/or T3 with a suppressed TSH. In this typical primary pattern, identification of the underlying etiology is crucial because treatment strategies differ for Graves disease, toxic nodular disease, and thyroiditis-related hormone leakage. A positive thyrotropin receptor antibody (TRAb) is highly supportive of Graves disease and can confirm an autoimmune mechanism in the appropriate biochemical setting.[17] However, not all thyrotoxicosis follows the classic pituitary feedback pattern. In pituitary tumor–related hyperthyroidism (TSHoma), TSH is usually elevated or inappropriately normal despite high thyroid hormone levels. Additional biochemical clues can include elevated sex hormone–binding globulin (a peripheral marker induced by thyroid hormone), an elevated alpha-subunit, and an increased alpha-subunit–to-TSH ratio, reflecting pituitary tumor secretory behavior. A different explanation for high or inappropriately normal TSH in the presence of elevated thyroid hormones is resistance to thyroid hormone (THR), typically an autosomal dominant condition in which target tissue responsiveness is reduced, disrupting expected feedback dynamics. In THR, the FT4:FT3 ratio may be reduced, and TRH stimulation often yields a normal or exaggerated TSH response, a pattern that helps differentiate it from pituitary tumor physiology when interpreted alongside imaging and clinical phenotype.[17] Clinically important “special causes” of thyrotoxicosis also include hCG-mediated states, such as hydatidiform mole or embryonal carcinoma, where markedly elevated hCG stimulates the TSH receptor and drives increased thyroid hormone production. These patients may present with low TSH, high T4 and T3, and elevated hCG, emphasizing the need to integrate endocrine testing with oncologic or obstetric evaluation. T3 toxicosis is another variant, in which T3 is elevated while T4 remains normal, accompanied by suppressed TSH; this pattern reinforces why FT3 measurement is sometimes necessary when TSH is low but FT4 is not elevated. Conversely, hyperthyroidism due to oral T3 administration may present with low T4 levels, an important clue to exogenous hormone exposure and a reminder that biochemical pattern recognition can prevent misclassification and inappropriate treatment.[17][40] Monitoring treated hyperthyroidism should include both TSH and FT4 because pituitary recovery is delayed and TSH may take months to normalize even when therapy is effective.[17][40]

Hypothyroidism

Hypothyroidism is most commonly caused by primary thyroid gland hypofunction, and the classic biochemical pattern is low T3 and T4 with elevated TSH. This pattern supports diagnosis, guides levothyroxine replacement, and allows dose titration to restore euthyroidism while avoiding overtreatment. In contrast, pituitary (central) hypothyroidism presents with low T3 and T4 but normal or low TSH, reflecting impaired pituitary output or altered TSH bioactivity. Recognition of this central pattern has major clinical implications: treatment is guided primarily by FT4 rather than TSH, and evaluation must extend beyond the thyroid to include pituitary structure and function, as concomitant adrenal insufficiency may coexist and must be addressed before or alongside thyroid hormone replacement.[40] In sum, the clinical significance of thyroid testing lies in its capacity to convert nonspecific symptoms into actionable diagnoses while preventing misinterpretation in contexts where TSH is insufficient.[17] Subclinical disease requires

risk-based decision-making, overt hyperthyroidism demands etiologic clarification and careful monitoring, and hypothyroidism—especially central forms—requires an interpretation strategy that prioritizes free hormone levels and broader endocrine assessment.[17][40]

Other Considerations

Interpretation of thyroid function tests becomes substantially more complex when physiological states, binding protein abnormalities, systemic illness, or medications distort the usual relationship between pituitary feedback (TSH) and circulating hormone concentrations. These "other considerations" are clinically significant because they can produce biochemical patterns that mimic thyroid disease or conceal it, leading to misdiagnosis, inappropriate treatment, or failure to recognize serious underlying pathology. For laboratory and endocrine teams, the central challenge is distinguishing true thyroid dysfunction from alterations in hormone transport, metabolism, and assay behavior while maintaining a patient-centered approach that integrates clinical context. A frequent source of interpretive confusion is variation in thyroid hormone–binding proteins, particularly thyroxine-binding globulin (TBG). Because total T4 and total T3 largely reflect hormone bound to transport proteins, increases in TBG commonly raise total hormone levels without increasing free hormone concentrations or producing symptoms of hyperthyroidism. Consequently, elevated total T4 and total T3 may be observed in patients with increased TBG, whereas isolated increases in total T4 may occur in familial binding abnormalities such as familial dysalbuminemic hyperthyroxinemia (FDH) or familial elevation of thyroid-binding prealbumin (transthyretin).[41] In these conditions, the patient can remain clinically euthyroid while laboratory values suggest "hyperthyroxinemia," underscoring why free hormone testing and careful pattern recognition are critical.

Hyperestrogenic states are among the most common causes of increased TBG. Pregnancy, estrogen-containing medications, and conditions associated with high estrogen activity—such as hydatidiform mole—can increase hepatic production of TBG, thereby elevating total T3 and total T4 while leaving TSH and free hormones relatively unchanged once physiologic equilibrium is established.[42] Beyond estrogen effects, increased TBG has also been associated with liver disease, lymphocytoma, acute intermittent porphyria, drug exposures (including heroin, clofibrate, methadone, and 5-fluorouracil), and acute psychosis, all of which can raise total hormone values and complicate interpretation when total assays are used indiscriminately.[42] Conversely, low TBG—whether congenital or induced by drugs such as androgens—reduces total T4 and total T3 while TSH remains normal, a pattern that can be mistakenly interpreted as hypothyroidism if the clinician relies on total hormone measurements rather than free hormone levels.[42] In practical terms, any unexpected discrepancy between total hormones and clinical status should trigger consideration of TBG status, medication history, pregnancy, and the possibility of inherited binding variants. Another important scenario is euthyroid hyperthyroxinemia, characterized by elevated T4 and T3 levels with a normal or mildly increased TSH, often linked to peripheral thyroid hormone resistance (THR).[43] In this setting, tissues respond less effectively to thyroid hormone, so pituitary feedback may not fully suppress TSH despite elevated circulating hormone levels. This biochemical pattern overlaps with that of a TSH-secreting pituitary adenoma (TSHoma), and careful differentiation is essential because management pathways diverge dramatically. While the full diagnostic workup extends beyond routine laboratory considerations, the key clinical lesson is that elevated thyroid hormones with non-suppressed TSH should not be reflexively labeled as assay error or dismissed as "subclinical hyperthyroidism"; rather, it demands systematic evaluation, often including repeat testing, assessment for interference, and endocrine-directed investigations.[43]

Systemic illness introduces another layer of complexity through nonthyroidal illness syndrome (euthyroid sick syndrome). In severely ill patients, this syndrome typically presents with low T3 and variable T4, which may be normal, reduced, or occasionally elevated, reflecting changes in deiodination, binding protein production, and hypothalamic signaling rather than intrinsic thyroid gland failure. Progression from nonthyroidal illness to true thyrotoxicosis is extremely rare, and low T4 and T3 values often correlate with poor prognosis, likely due to reduced hepatic production of T4 and TBG in critical illness. Pharmacologic exposures in the intensive care environment further influence this pattern: dopamine can inhibit the TSH response to thyroid-releasing hormone, lowering measured TSH, while furosemide can inhibit T4 binding and shift measured hormone fractions. During recovery, if the patient survives the acute illness, TSH levels often rise transiently, which can be misread as developing hypothyroidism unless interpreted in the context of convalescence. This dynamic trajectory illustrates why routine thyroid screening in critical illness can be misleading and why repeat testing after recovery is often more informative than reflex treatment during acute illness. Acute myocardial infarction represents a specific illness state with characteristic temporal changes in thyroid hormones. Biochemical alterations can include low T3 levels that peak around three days after infarction and higher T4 levels peaking approximately six to seven days post-event.[44] These shifts likely reflect acute stress physiology and

altered peripheral conversion rather than primary thyroid pathology. Clinically, this matters because thyroid tests drawn during acute coronary events may not reflect the patient's baseline thyroid state and should be interpreted cautiously, especially when results are borderline and the clinical goal is cardiovascular stabilization [42][43][44].

Medication effects extend far beyond TBG alterations and frequently influence thyroid hormone metabolism and binding in ways that reshape laboratory profiles. Propranolol, for example, blocks peripheral conversion of T4 to T3, leading to elevated T4 and reduced T3—an effect that can be therapeutically beneficial in thyrotoxicosis but can also complicate interpretation if a clinician expects parallel movement of T4 and T3.[45][46] Amiodarone and iodine-containing contrast agents (including ipodate and iopanoate) also impair peripheral conversion of T4 to T3 and exert additional, less predictable effects on thyroid physiology, sometimes precipitating hypo- or hyperthyroidism depending on underlying thyroid status and iodine handling.[45][46] Several drugs displace T4 from binding proteins, including heparin, salicylates, diazepam, fenclofenac, and phenylbutazone, which can reduce measured total T4; salicylates and fenclofenac may also lower total T3.[45][46] Diphenylhydantoin (phenytoin) both displaces T4 from binding proteins and accelerates T4 clearance via hepatic enzyme induction, combining distributional and metabolic mechanisms that can lower total hormone levels and potentially distort free hormone estimates depending on assay platform.[47] Additional drug classes influence hormone synthesis or clearance: sulfonylureas and sulfonamides can interfere with organification and thyroid hormone synthesis, and sulfonylureas may further displace T4 from TBG, potentially contributing to hypothyroidism in susceptible patients.[48] Enzyme-inducing anticonvulsants such as carbamazepine and phenobarbital increase T4 clearance, thereby lowering circulating hormone levels without necessarily indicating intrinsic thyroid gland failure.[49] Sodium nitroprusside has been associated with inhibition of thyroid function and reduced T4 levels, while heavy cigarette smoking has been linked to mild reductions in total T4, total T3, and FT4, and phenothiazine use has been associated with lower total T4.[45][46] Renal dysfunction is another condition in which thyroid testing may appear abnormal despite complex pathophysiology rather than primary thyroid disease. Patients with impaired kidney function may show low T3 and T4 levels with normal or high TSH, reflecting altered hormone metabolism, protein binding, and clearance dynamics.[50] Endocrine pituitary pathology can also reshape thyroid tests indirectly. Prolactin-secreting tumors may suppress TSH, producing low T4 with inappropriately normal TSH, potentially due to increased dopamine activity that suppresses pituitary TSH secretion. In addition, certain metabolic and endocrine disorders can create distinctive but uncommon biochemical associations. Low total T4 and total T3 with low FT3 and elevated TSH have been described in pseudohypoparathyroidism type 1, illustrating how broader endocrine dysregulation can influence thyroid-related laboratories and emphasizing the need for integrated endocrine evaluation when patterns do not fit standard thyroid disease models [44][45][46].

Finally, low T3 states deserve special attention because they are prevalent and easily misinterpreted. Decreased deiodination of T4 to T3 can occur in protein-energy malnutrition, poorly controlled diabetes mellitus, and in the setting of multiple drugs—including propylthiouracil, glucocorticoids, iopanoate, sodium ipodate, amiodarone, and colistipal—as well as in severely ill patients. Low T3 may also be observed in hyperparathyroidism and with propylthiouracil use, reflecting both disease-related and treatment-related effects on peripheral conversion.[51] The clinical implication is that an isolated low T3 does not automatically signify hypothyroidism; instead, it often signals altered peripheral metabolism and should prompt assessment of nutritional status, systemic illness severity, medication exposure, and the broader endocrine context. Collectively, these considerations highlight that thyroid tests are not interpreted in a vacuum. Alterations in binding proteins can elevate or depress total hormone values without changing thyroid function, euthyroid hyperthyroxinemia and THR can produce paradoxical feedback patterns, critical illness can drive adaptive changes that mimic thyroid disease, and multiple medications can alter binding, conversion, synthesis, and clearance.[41][42][43][44][45][46][47][48][49][50][51] Safe interpretation therefore requires contextual reporting, careful review of exposures and comorbidities, and, when necessary, confirmatory testing using alternative methods or repeat measurement after recovery from transient physiologic stressors.

Quality Control and Lab Safety

A quality management system (QMS) is fundamental to ensuring reliable thyroid function testing (TFT), enabling accurate and consistent measurement of TSH, FT4, and T3 across diverse clinical contexts and over time. Because TFTs frequently guide high-impact decisions—such as initiating lifelong hormone replacement, titrating antithyroid therapy, or monitoring thyroid cancer patients—analytical errors can translate directly into misdiagnosis, inappropriate treatment escalation, or delayed recognition of critical illness. Within this framework, quality control (QC) serves as the operational backbone of the QMS, functioning as a continuous safeguard against analytical drift, reagent failure, instrument malfunction, and process

variability. A robust QMS therefore integrates policies, standardized procedures, personnel competency, equipment oversight, and documentation practices to ensure that results are reproducible, traceable, and clinically trustworthy.[52] QC in thyroid testing is typically structured around two complementary pillars: internal quality control and external quality assessment. Internal QC is performed daily—often multiple times per run—by analyzing control materials with known target values in parallel with patient specimens. Control results are plotted on Levey–Jennings charts, which provide a visual representation of assay stability over time and allow laboratories to detect trends before they reach clinically significant magnitude. Laboratories then apply Westgard rules to interpret QC performance, using predefined statistical criteria to decide whether a run is acceptable or must be rejected. These rules support differentiation between random error, which manifests as unpredictable variation (for example, pipetting inconsistency, transient instrument aspiration issues, or sporadic sample probe interference), and systematic error, which appears as consistent bias (for example, gradual calibration drift, reagent degradation, temperature instability, or optical system deterioration).[53] This distinction is clinically meaningful because it shapes the corrective pathway: random errors require rapid containment and immediate process verification, whereas systematic errors demand deeper root-cause analysis and intervention to prevent sustained release of biased patient results [52][53].

When random error is identified, laboratories must respond promptly to avoid reporting unreliable values. Corrective steps may include repeating controls, recalibrating the analyzer, inspecting pipetting systems, verifying reagent loading and expiration, and ensuring procedural consistency across staff. Human factors contribute substantially to random error, so retraining, competency reassessment, and workload redesign may be necessary when repeated failures occur. Preventive strategies, such as automation of sample handling, barcode-based reagent tracking, and standardized run sequencing, reduce operator variability and improve the stability of high-throughput thyroid assays. In contrast, systematic errors often require interventions that address underlying bias drivers, including replacing reagent lots, performing fresh calibration with validated calibrators, confirming instrument temperature control, and scheduling maintenance for aging components. Because systematic drift can persist unnoticed until clinical anomalies emerge, proactive monitoring is essential, and laboratories should treat subtle trend shifts in QC plots as early warning signals rather than waiting for a full Westgard rule violation.[54] External QC—commonly described as proficiency testing or external quality assessment—adds a necessary layer of independent verification. In this process, laboratories receive blind samples from an external organization and analyze them using routine workflows. The reported results are then compared with peer laboratories and, where applicable, with reference-method targets. This benchmarking is particularly valuable in thyroid testing because different immunoassay platforms can vary in calibration and susceptibility to interferences, meaning a laboratory may appear internally stable while still being systematically biased compared with broader consensus. External QC helps reveal such platform-specific or laboratory-specific biases that internal controls may not detect, thereby strengthening confidence that reported TSH, FT4, and antibody results align with industry expectations and are comparable across institutions.[55]

Beyond formal internal and external QC, advanced laboratories often incorporate patient-based quality control as an additional safeguard. This approach leverages aggregate patient data to detect unexpected shifts in analyte distributions, which can indicate emerging assay issues. For example, a sudden population-wide increase in measured TSH or a disproportionate rise in suppressed TSH results may suggest calibration error, reagent lot instability, or analyzer malfunction. Similarly, unexpected shifts in FT4 distributions—particularly when not mirrored by changes in TSH—may raise suspicion for methodological drift or reagent changes. Patient-based monitoring does not replace control testing, but it complements it by providing real-world confirmation that assay performance remains stable within the clinical population being served. When integrated thoughtfully, this surveillance can identify subtle problems early and reduce the likelihood that analytical issues persist long enough to affect large numbers of patients.[56] Documentation is the connective tissue that makes quality systems auditable, reproducible, and improvable. Laboratories must meticulously record control outcomes, lot numbers, calibration events, corrective actions, instrument maintenance, and staff competency activities. These records provide traceability, allowing laboratories to retrospectively evaluate whether a patient result may have been affected by a known QC event, and they support regulatory compliance during inspections and accreditation audits. Routine reagent validation—especially when new lots are introduced—helps ensure continuity of performance and reduces the risk of lot-to-lot bias, which is a well-recognized issue in immunoassay-based testing. Likewise, scheduled preventive maintenance and verification of analyzer performance characteristics reinforce reliability by reducing unexpected downtime and preventing gradual component degradation from undermining analytical accuracy. In a mature QMS, documentation is not merely administrative; it is a structured dataset

used to drive continuous improvement and risk reduction.[57]

Technological advances have strengthened QC capacity by embedding automated checks into analyzer workflows. Modern platforms can detect clotting, sample aspiration failure, reagent volume anomalies, and calibration drift in near real time, prompting operator intervention before results are released. Some systems implement built-in delta checks or flagging rules that compare new results with prior patient values, especially for monitored therapies, thereby reducing the chance that a spurious TSH or FT4 result is reported without review. However, technology does not eliminate the need for skilled oversight. Competent personnel must interpret flags appropriately, recognize patterns suggestive of interference, and escalate concerns when biochemical profiles appear internally inconsistent. Ongoing education and competency-based training remain central to maintaining quality, particularly as assays evolve and new sources of interference—such as biotin supplementation or novel monoclonal antibody therapies—become more common in the patient population. Training also reinforces adherence to standardized operating procedures and fosters a culture in which quality and patient safety are shared responsibilities rather than isolated tasks.[58] Laboratory safety is inseparable from quality because unsafe practices increase the risk of specimen contamination, staff injury, workflow disruption, and legal or regulatory failure. Facilities conducting TFTs routinely handle blood specimens that may contain bloodborne pathogens, including HIV and hepatitis viruses, making strict adherence to biosafety principles essential. Personnel should use appropriate personal protective equipment (PPE), including gloves, laboratory coats, and eye or face protection when splash risk exists. Safe venipuncture and specimen handling procedures reduce exposure risk, and all sharps must be disposed of immediately into approved sharps containers to prevent needlestick injuries. Accurate labeling and correct storage conditions protect specimen integrity and reduce pre-analytical error, which is a major contributor to incorrect thyroid results. When testing is delayed, refrigeration of specimens—as guided by validated stability data—helps preserve analyte performance and prevents degradation-related variability.[59]

Chemical and instrument safety considerations are also significant. Immunoassay reagents may include preservatives, buffers, and chemicals that require controlled storage and handling in accordance with manufacturer recommendations. Volatile or irritating substances should be handled in appropriate ventilation conditions, including fume hoods when indicated, and staff must be trained in reading safety data sheets and recognizing chemical exposure risks. Automated analyzers introduce mechanical, electrical, and ergonomic hazards; therefore, only trained personnel should operate, troubleshoot, and maintain these systems. Regular calibration and maintenance reduce both analytical errors and malfunction-related safety incidents, such as fluid leaks, overheating, or electrical faults. Waste management is another safety-critical domain: biohazardous waste (including blood tubes and contaminated consumables) and chemical waste must be segregated and disposed of according to local regulations to prevent environmental contamination and occupational exposure. Emergency readiness—spill kits, eyewash stations, first-aid supplies, and exposure protocols—must be maintained and rehearsed so that accidents are addressed promptly and effectively. Staff training in incident response is essential, as rapid containment of spills or exposure events protects both personnel and continuity of laboratory operations.[60] In summary, reliable TFT delivery depends on an integrated QMS that combines internal QC, external proficiency testing, patient-based monitoring, rigorous documentation, and continuous personnel development.[52][53][54][55][56][57][58] Equally, safe laboratory practice protects staff and preserves specimen integrity through PPE, sharps safety, correct storage, controlled reagent handling, instrument maintenance, compliant waste disposal, and emergency preparedness.[59][60] Together, these quality and safety systems ensure that thyroid laboratory results remain dependable foundations for endocrine decision-making and patient care.

Conclusion:

Thyroid function testing is a cornerstone of endocrine diagnostics, yet its reliability depends on more than analytical precision. The complexity of thyroid physiology, combined with numerous interfering factors, demands a systematic approach that integrates laboratory rigor with clinical reasoning. While TSH serves as an effective initial screen, exclusive reliance on this marker can lead to missed diagnoses in central hypothyroidism, treatment transitions, and non-thyroidal illness. Free hormone measurements, antibody testing, and confirmatory methods such as equilibrium dialysis or mass spectrometry are indispensable in resolving discordant patterns and ensuring diagnostic accuracy. Equally important is the recognition of pre-analytical and analytical vulnerabilities—ranging from specimen handling errors to heterophile antibody interference—that can produce misleading results. A robust quality management system, incorporating internal and external QC, patient-based monitoring, and thorough documentation, safeguards against these risks and supports consistent performance across platforms. Ultimately, thyroid testing should never be interpreted in isolation. Clinical context—including comorbid conditions, medication exposure, and physiologic states such as pregnancy—must guide interpretation to prevent overtreatment or underdiagnosis. By combining technical excellence with contextual awareness, laboratories and clinicians

can transform thyroid function testing into a reliable foundation for patient-centered endocrine care.

References:

1. Brochmann H, Bjøro T, Gaarder PI, Hanson F, Frey HM. Prevalence of thyroid dysfunction in elderly subjects. A randomized study in a Norwegian rural community (Naerøy). Acta endocrinologica. 1988 Jan:117(1):7-12
2. Feingold KR, Ahmed SF, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, de Herder WW, Dhatariya K, Dungan K, Hofland J, Kalra S, Kaltsas G, Kapoor N, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, Muzumdar R, Purnell J, Rey R, Sahay R, Shah AS, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP, Spencer CA. Assay of Thyroid Hormones and Related Substances. Endotext. 2000
3. Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. Best practice & research. Clinical endocrinology & metabolism. 2013 Dec:27(6):745-62. doi: 10.1016/j.beem.2013.10.003.
4. Rivas AM, Lado-Abeal J. Thyroid hormone resistance and its management. Proceedings (Baylor University. Medical Center). 2016 Apr:29(2):209-11
5. Pearce EN, Farwell AP, Braverman LE. Thyroiditis. The New England journal of medicine. 2003 Jun 26:348(26):2646-55
6. Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. Autoimmunity reviews. 2015 Feb:14(2):174-80. doi: 10.1016/j.autrev.2014.10.016.
7. Roberts CG, Ladenson PW. Hypothyroidism. Lancet (London, England). 2004 Mar 6:363(9411):793-803
8. Franklyn JA, Boelaert K. Thyrotoxicosis. Lancet (London, England). 2012 Mar 24:379(9821):1155-66. doi: 10.1016/S0140-6736(11)60782-4.
9. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid : official journal of the American Thyroid Association. 2016 Jan:26(1):1-133. doi: 10.1089/thy.2015.0020.
10. Tanner M, Kent N, Smith B, Fletcher S, Lewer M. Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation. Annals of clinical biochemistry. 2008 Jul:45(Pt 4):375-9. doi: 10.1258/acb.2007.007183.
11. Mardell R, Gamlen TR. Discrepant results for free thyroxin by radioimmunoassay and dialysis procedures explained. Clinical chemistry. 1982 Sep:28(9):1989
12. Ercan M, Fırat Oğuz E, Akbulut ED, Yilmaz M, Turhan T. Comparison of the effect of gel used in two different serum separator tubes for thyroid function tests. Journal of clinical laboratory analysis. 2018 Jul:32(6):e22427. doi: 10.1002/jcla.22427.
13. Keffer JH. Preanalytical considerations in testing thyroid function. Clinical chemistry. 1996 Jan:42(1):125-34
14. Philippe J, Dibner C. Thyroid circadian timing: roles in physiology and thyroid malignancies. Journal of biological rhythms. 2015 Apr:30(2):76-83. doi: 10.1177/0748730414557634.
15. Soldin OP, Chung SH, Colie C. The Use of TSH in Determining Thyroid Disease: How Does It Impact the Practice of Medicine in Pregnancy? Journal of thyroid research. 2013:2013():148157. doi: 10.1155/2013/148157.
16. Fisher DA. Physiological variations in thyroid hormones: physiological and pathophysiological considerations. Clinical chemistry. 1996 Jan:42(1):135-9
17. Soh SB, Aw TC. Laboratory Testing in Thyroid Conditions - Pitfalls and Clinical Utility. Annals of laboratory medicine. 2019 Jan:39(1):3-14. doi: 10.3343/alm.2019.39.1.3.
18. Spencer CA, Schwarzbein D, Guttler RB, LoPresti JS, Nicoloff JT. Thyrotropin (TSH)-releasing hormone stimulation test responses employing third and fourth generation TSH assays. The Journal of clinical endocrinology and metabolism. 1993 Feb:76(2):494-8
19. Van Houcke SK, Van Uytfanghe K, Shimizu E, Tani W, Umemoto M, Thienpont LM. IFCC international conventional reference procedure for the measurement of free thyroxine in serum: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group for Standardization of Thyroid Function Tests (WG-STFT)(1). Clinical chemistry and laboratory medicine. 2011 Aug:49(8):1275-1281. doi: 10.1515/CCLM.2011.639.
20. Soukhova N, Soldin OP, Soldin SJ. Isotope dilution tandem mass spectrometric method for T4/T3. Clinica chimica acta; international journal of clinical chemistry. 2004 May:343(1-2):185-90
21. Thienpont LM, Van Uytfanghe K, Marriott J, Stokes P, Siekmann L, Kessler A, Bunk D, Tai S. Feasibility study of the use of frozen human sera

in split-sample comparison of immunoassays with candidate reference measurement procedures for total thyroxine and total triiodothyronine measurements. Clinical chemistry. 2005 Dec:51(12):2303-11

22. Thienpont LM, Van Uytfanghe K, Beastall G, Faix JD, Ieiri T, Miller WG, Nelson JC, Ronin C, Ross HA, Thijssen JH, Toussaint B, IFCC Working Group on Standardization of Thyroid Function Tests. Report of the IFCC Working Group for Standardization of Thyroid Function Tests; part 3: total thyroxine and total triiodothyronine. Clinical chemistry. 2010 Jun:56(6):921-9. doi: 10.1373/clinchem.2009.140228.
23. Ross HA, Menheere PP, Endocrinology Section of SKML (Dutch Foundation for Quality Assessment in Clinical Laboratories), Thomas CM, Mudde AH, Kouwenberg M, Wolffenbuttel BH. Interference from heterophilic antibodies in seven current TSH assays. Annals of clinical biochemistry. 2008 Nov:45(Pt 6):616. doi: 10.1258/acb.2008.008066.
24. Zouwail SA, O'Toole AM, Clark PM, Begley JP. Influence of thyroid hormone autoantibodies on 7 thyroid hormone assays. Clinical chemistry. 2008 May:54(5):927-8. doi: 10.1373/clinchem.2007.099770.
25. Sapin R, Gasser F, Schlienger JL. Familial dysalbuminemic hyperthyroxinemia and thyroid hormone autoantibodies: interference in current free thyroid hormone assays. Hormone research. 1996:45(3-5):139-41
26. Ohba K, Noh JY, Unno T, Satoh T, Iwahara K, Matsushita A, Sasaki S, Oki Y, Nakamura H. Falsely elevated thyroid hormone levels caused by anti-ruthenium interference in the Elecsys assay resembling the syndrome of inappropriate secretion of thyrotropin. Endocrine journal. 2012:59(8):663-7
27. Loh TP, Kao SL, Halsall DJ, Toh SA, Chan E, Ho SC, Tai ES, Khoo CM. Macro-thyrotropin: a case report and review of literature. The Journal of clinical endocrinology and metabolism. 2012 Jun:97(6):1823-8. doi: 10.1210/jc.2011-3490.
28. Cartwright D, O'Shea P, Rajanayagam O, Agostini M, Barker P, Moran C, Macchia E, Pinchera A, John R, Agha A, Ross HA, Chatterjee VK, Halsall DJ. Familial dysalbuminemic hyperthyroxinemia: a persistent diagnostic challenge. Clinical chemistry. 2009 May:55(5):1044-6. doi: 10.1373/clinchem.2008.120303. E
29. Stockigt JR, Lim CF. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine. Best practice & research. Clinical endocrinology & metabolism. 2009 Dec:23(6):753-67. doi: 10.1016/j.beem.2009.06.004.
30. Stevenson HP, Archbold GP, Johnston P, Young IS, Sheridan B. Misleading serum free thyroxine results during low molecular weight heparin treatment. Clinical chemistry. 1998 May:44(5):1002-7
31. Després N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clinical chemistry. 1998 Mar:44(3):440-54
32. Krasowski MD. Educational Case: Hemolysis and Lipemia Interference With Laboratory Testing. Academic pathology. 2019 Jan-Dec:6():2374289519888754. doi: 10.1177/2374289519888754.
33. Clark PM, Holder RL, Haque SM, Hobbs FD, Roberts LM, Franklyn JA. The relationship between serum TSH and free T4 in older people. Journal of clinical pathology. 2012 May:65(5):463-5. doi: 10.1136/jclinpath-2011-200433.
34. Koulouri O, Gurnell M. How to interpret thyroid function tests. Clinical medicine (London, England). 2013 Jun:13(3):282-6. doi: 10.7861/clinmedicine.13-3-282.
35. Hoermann R, Eckl W, Hoermann C, Larisch R. Complex relationship between free thyroxine and TSH in the regulation of thyroid function. European journal of endocrinology. 2010 Jun:162(6):1123-9. doi: 10.1530/EJE-10-0106.
36. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C, Cooper RS, Weissman NJ. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA. 2004 Jan 14:291(2):228-38
37. Gurnell M, Halsall DJ, Chatterjee VK. What should be done when thyroid function tests do not make sense? Clinical endocrinology. 2011 Jun:74(6):673-8. doi: 10.1111/j.1365-2265.2011.04023.x.
38. Ylli D, Klubo-Gwiezdzinska J, Wartofsky L. Thyroid emergencies. Polish archives of internal medicine. 2019 Aug 29:129(7-8):526-534. doi: 10.20452/pamw.14876.
39. Popoveniuc G, Chandra T, Sud A, Sharma M, Blackman MR, Burman KD, Mete M, Desale S, Wartofsky L. A diagnostic scoring system for myxedema coma. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2014 Aug:20(8):808-17. doi: 10.4158/EP13460.OR.
40. Walker HK, Hall WD, Hurst JW, Dunlap DB. Thyroid Function Tests. Clinical Methods: The History, Physical, and Laboratory Examinations. 1990
41. Ting MJM, Zhang R, Lim EM, Ward BK, Wilson SG, Walsh JP. Familial Dysalbuminemic Hyperthyroxinemia as a Cause for Discordant

Thyroid Function Tests. Journal of the Endocrine Society. 2021 Apr 1:5(4):bvab012. doi: 10.1210/jendso/bvab012.
42. Díaz S, Cárdenas H, Brandeis A, Miranda P, Salvatierra AM, Croxatto HB. Relative contributions of anovulation and luteal phase defect to the reduced pregnancy rate of breastfeeding women. Fertility and sterility. 1992 Sep:58(3):498-503
43. Olateju TO, Vanderpump MP. Thyroid hormone resistance. Annals of clinical biochemistry. 2006 Nov:43(Pt 6):431-40
44. Rasool R, Unar A, Jafar TH, Chanihoon GQ, Mubeen B. A Role of Thyroid Hormones in Acute Myocardial Infarction: An Update. Current cardiology reviews. 2023:19(1):e280422204209. doi: 10.2174/1573403X18666220428121431.
45. Nademanee K, Piwonka RW, Singh BN, Hershman JM. Amiodarone and thyroid function. Progress in cardiovascular diseases. 1989 May-Jun:31(6):427-37
46. Wiersinga WM. Propranolol and thyroid hormone metabolism. Thyroid : official journal of the American Thyroid Association. 1991 Summer:1(3):273-7
47. Heyma P, Larkins RG, Perry-Keene D, Peter CT, Ross D, Sloman JG. Thyroid hormone levels and protein binding in patients on long-term diphenylhydantoin treatment. Clinical endocrinology. 1977 May:6(5):369-76
48. Hershman JM, Craane TJ, Colwell JA. Effect of sulfonylurea drugs on the binding of triiodothyronine and thyroxine to thyroxine-binding globulin. The Journal of clinical endocrinology and metabolism. 1968 Nov:28(11):1605-10
49. Rootwelt K, Ganes T, Johannessen SI. Effect of carbamazepine, phenytoin and phenobarbitone on serum levels of thyroid hormones and thyrotropin in humans. Scandinavian journal of clinical and laboratory investigation. 1978 Dec:38(8):731-6
50. Mohamedali M, Reddy Maddika S, Vyas A, Iyer V, Cheriyath P. Thyroid disorders and chronic kidney disease. International journal of nephrology. 2014:2014():520281. doi: 10.1155/2014/520281.
51. Heinen E, Herrmann J, Mosny D, Moreno F, Teschke R, Krüskemper HL. Inhibition of peripheral deiodination of 3, 5, 3'-triiodothyronine: an adverse effect of propylthiouracil in the treatment of T3-thyrotoxicosis. Journal of endocrinological investigation. 1981 Jul-Sep:4(3):331-4
52. Zhang S, Wang W, Zhao H, He F, Zhong K, Yuan S, Wang Z. Status of internal quality control for thyroid hormones immunoassays from 2011 to 2016 in China. Journal of clinical laboratory analysis. 2018 Jan:32(1):. doi: 10.1002/jcla.22154.
53. Hu LT, Wang ZG. Internal quality control practice of thyroid disease related tests and imprecision analysis in China. Clinical laboratory. 2014:60(2):301-8
54. Kinns H, Pitkin S, Housley D, Freedman DB. Internal quality control: best practice. Journal of clinical pathology. 2013 Dec:66(12):1027-32. doi: 10.1136/jclinpath-2013-201661.
55. James D, Ames D, Lopez B, Still R, Simpson W, Twomey P. External quality assessment: best practice. Journal of clinical pathology. 2014 Aug:67(8):651-5. doi: 10.1136/jclinpath-2013-201621.
56. Zhang Y, Wang HL, Xie YH, He DH, Zhou CQ, Kong LR. Practical application of the patient data-based quality control method: the potassium example. Biochemia medica. 2024 Feb 15:34(1):010901. doi: 10.11613/BM.2024.010901.
57. Valenstein PN, Stankovic AK, Souers RJ, Schneider F, Wagar EA. Document control practices in 120 clinical laboratories. Archives of pathology & laboratory medicine. 2009 Jun:133(6):942-9
58. Wadhwa V, Rai S, Thukral T, Chopra M. Laboratory quality management system: road to accreditation and beyond. Indian journal of medical microbiology. 2012 Apr-Jun:30(2):131-40. doi: 10.4103/0255-0857.96647.
59. Lunn G, Lawler Chemical Resistance Of Gloves G. Laboratory safety. Current protocols in protein science. 2002 Aug:Appendix 2():A.2A.1-A.2A.35. doi: 10.1002/0471140864.psa02as28.
1. Tait FN, Mburu C, Gikunju J. Occupational safety and health status of medical laboratories in Kajiado County, Kenya. The Pan African medical journal. 2018:29():65. doi: 10.11604/pamj.2018.29.65.12578