Preanalytical considerations in testing thyroid function

JOSEPH H. KEFFER

Remarkable technical advances have permitted analytical measurement of thyrotropin (TSH) and estimates of free thyroxine (FT_{4}) with precision, accuracy, and favorable economics. Combined with an increased appreciation of the key insights into the pituitary-thyroid relation, preanalytical considerations infrequently introduce confounding variables. In reviewing thyroid data, preanalytical considerations include physiological and specimen-based issues. Central to the improvement in thyroid assessment is the recognition that physiological individuals maintain their FT_{4} within narrow limits. When this deviates, there is a logarithmic response of the TSH concentration to the arithmetic shift in FT_{4}. In effect, the TSH deviation magnifies the subtle shift in FT_{4}. Artifact and other nonthyroid-related preanalytical considerations are infrequently the cause of nonconcordance when discrepancy occurs between the reported values for FT_{4} and TSH. When abnormalities of TSH and FT_{4} are encountered, the probability strongly favors a disease state rather than a preanalytical variable. Infrequent but real extrathyroidal pathophysiological states are increasingly recognized as a result of the reliable assessment of the pituitary-thyroid relation.

INDEXING TERMS: preanalytical variables • thyrotropin • free thyroxine • variation, source of

Through routine sensitive measurement of thyrotropin (TSH) and estimates of free thyroxine (FT_{4}), the assessment of thyroid function has become a mature field.\textsuperscript{1} In 1988, Gorman addressed a group of thyroidologists with regard to the contribution of these newer sensitive assays for measuring serum TSH concentration. He stated: "... measurements of thyroid hormone in the blood have been superseded by a new generation of thyrotropin ... assays that are so consistent, sensitive, and reliable that they render obsolete many of our previous practices and procedures\textsuperscript{2} [1]."

Three concepts are integral to understanding current advances in the routine assessment of thyroid function. Each should be considered in the broad view of preanalytical considerations. First, the underlying basis of the TSH/FT_{4} diagnostic strategy is the narrow intra-individual variation of FT_{4} and the associated log-linear relation of TSH to changes of FT_{4}. Second, in contrast to the T_{4}-based testing strategies, preanalytical variables infrequently and usually insignificantly affect the TSH/FT_{4} testing strategy in terms of medical relevance. Third, technological advances in analytical methods permit convenient and economical measurement of TSH and FT_{4}.

Preanalytical artifact is infrequent and creates minimal impact on diagnostic testing. In the context of pragmatic clinical application, there is a clear consensus among thyroidologists in favor of the TSH/FT_{4} strategy [2-4]. In fact, the rare reported dissent is notable by isolated exception [5].

This presentation will focus on preanalytical considerations in thyroid-function testing, with primary emphasis on measurement of TSH and FT_{4} rather than the 22 tests currently described as appropriate by Lindsred et al. [6]. Clearly, the robust nature of these two dominant assays underscores the relatively minimal impact of routine preanalytical variables on these tests, in contrast to the significant variation in test results attributable to disease. Most of these variables are of interest for the study of subtle differences in physiological or research studies rather than for clinical diagnosis or routine monitoring. Significant preanalytical variables, when encountered, are almost always readily recognized as measurable TSH inappropriately present in definite thyrotoxic states. They often expose clinically relevant underlying pathophysiology, in contrast to the frequently confounding variations of thyroid-binding proteins associated with T_{4}-based diagnostic strategies. Indeed, current reviews regarding thyroid assessment and therapy are notable by virtue of their silence or minimal emphasis on preanalytical variables, and appropriately so.

For the purpose of this presentation, I have proposed a classification of preanalytical variables that is clinically based (Table 1), in keeping with some earlier classifications of biochemical testing results [7, 8]. As such, I have attempted to emphasize the importance of integrating all aspects contributing
Table 1: Classification of preanalytical variables in assessment of thyroid function.

<table>
<thead>
<tr>
<th>Physiological</th>
<th>iatrogenic causes</th>
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<tr>
<td>Set point for T4</td>
<td>Prior thyroid disease treatment</td>
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<tr>
<td>Log-linear relation of TSH/FT4</td>
<td>Surgical</td>
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<td>Seasonal influences</td>
<td>Drug therapy</td>
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<td>Environmental</td>
<td>Systemic</td>
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<td>Exercise</td>
<td>Topical</td>
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<td>Posture and immobilization</td>
<td>Plasmapheresis</td>
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<td>Pathophysiological</td>
<td>Specimen-based variables</td>
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<td>TSH-independent states</td>
<td>Stability and storage</td>
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<td>Common</td>
<td>Lipemia</td>
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<td>Rare</td>
<td>Stasis</td>
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<td>TSH-dependent states</td>
<td>Hemolysis</td>
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<td>Pituitary adenoma</td>
<td>Icterus</td>
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<td>Resistance states</td>
<td>Assay susceptibility</td>
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<td>Generalized</td>
<td>Antibodies</td>
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<td>Selective organ resistance</td>
<td>Heterophile</td>
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<td>TSH-independent states</td>
<td>Autoimmune</td>
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<td>Generalized</td>
<td>Protein binding</td>
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<td>Pituitary</td>
<td>Human error</td>
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<td>Psychiatric states</td>
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<td>Smoking</td>
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<td>Compliance</td>
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Table 2: Evidence for individual set point for T4.

| Stable intraindividual values over time. |
| Limited circadian amplitude of variation. |
| Narrow range relative to reference populations. |
| Log TSH response to linear changes of T4 or FT4. |
| Iodide administration lowering T4, raising TSH. |
| Intraiindvidual reproducibility of TRH response. |
placement therapy, the same basic principles relating to set point and log-linear response permit precise modulation of TSH concentrations, which define appropriate replacement therapy [4, 18]. The goal of therapy is restoration of physiological TSH concentrations [18]. Hershman et al. recommend application of the same TSH range for elderly persons as for younger individuals [28].

Although the pituitary response to fluctuations in FT₄ is our usual guide to the euthyroid state, we should recognize variable organ response to T₄ and triiodothyronine (T₃) concentrations. This is demonstrated in states of pituitary resistance, with varying responses of heart, liver, and muscle, which testify to the significance of nuclear receptor studies revealing variable binding of T₄ and T₃ in these tissues [18]. We may anticipate a more sophisticated definition of euthyroidism to evolve that will be based not solely on pituitary response but on various end organ responses as well.

**Circadian Rhythm**

Although a well-established TSH peak occurs in the late evening and a nadir at about midday [29–32], the amplitude of this fluctuation is small, with a mean of ~0.95 mIU/L [31] and ~2.0 mIU/L in a more recent study [32]. Custro and Scaglione (30) observed single individuals with a fluctuation from a low of 2.3 to a peak of 11.2 mIU/L. Although unlikely to create confusion as a preanalytical variable, the loss of the evening surge is correlated with central hypothyroidism [32] and may be diagnostically useful. The appearance of detectable TSH only during the nighttime surge proved that exogenous suppression was incomplete [29]. TRH response correlates better with nocturnal than with daytime TSH concentrations [29, 32].

**Seasonal Influences**

Seasonal and annual fluctuations of TSH, TT₄, FT₄, and TT₃ are minor. Although of interest physiologically, the shift is insufficient to reassign an individual from the euthyroid diagnostic category to an abnormal one [22]. Again emphasizing the sensitivity of the hypothalamic–pituitary–thyroid connection, peak TRH response correlates with peak seasonal TSH [22]. Seasonal fluctuation should be considered in assessing pregnant patients because they are followed longitudinally [21].

**Environmental Influences**

Exposure to cold temperature is generally associated with adaptive mechanisms. However, these are complex, relating to the duration and severity of the acute or chronic exposure as well as to numerous other variables [33], not the least of which is concomitant work effort and feeding status [34]. Observed changes are unlikely to create significant preanalytical influence on patient testing under the usual environmental conditions in North America experienced by the general population.

**Exercise**

Investigation of the pituitary–thyroid relation in women who participate in vigorous athletic activity with associated amenorrhea reveals no intrinsic disruption of thyroid function. However, the low-T₁ syndrome observed appears related to inadequate caloric intake [35]. This has been reversed and prevented by appropriate increased dietary energy intake [36]. Others studying prolonged exercise in training athletes report no consistent pattern with regard to the influence of exercise on thyroid-function status [37, 38]. After an acute episode of exercise, the experimentally predicted hourly pattern of TT₄ was altered but not TT₃ over the subsequent 8-h recovery phase [39]. Consequently, diagnostically significant alterations due to exercise are not a common and relevant preanalytical variable.

**Posture and Immobilization**

Although long known to affect hematocrit and constituents of the blood that do not leave the circulation under hydrostatic forces [40], little reference is made to the impact of posture and immobilization on thyroid hormones. Since T₄ and T₃ are 99.9% and 99.7% protein-bound, respectively [41], this variable is operative. TT₄ was observed to increase by a mean of 6.8% after subjects stood for 40 min [42].

**Pathophysiological Influences**

Common thyroid diseases presenting with functional deviations are TSH independent. In routinely encountered hypothyroidism and hyperthyroidism, the relation between TSH and FT₄ is the expected one, and they are addressed elsewhere. Preanalytical variables do not ordinarily affect this relation, especially in an outpatient population. On the other hand, nonthyroidal illness can alter the individual response and confound diagnosis. This subject is also addressed elsewhere.

For the sake of completeness, two pathological conditions are presented in this discussion of preanalytical variation. The first, trophoblastic disease, produces human chorionic gonadotropin (HCG), which normally has weak thyrotropic potency [43]. Some trophoblastic tumors produce modifications of the protein molecule with partial sialylation, which apparently leads to greater binding to the TSH receptor [43]. In conjunction with the very high concentrations seen in neoplastic proliferation, hyperthyroidism results [28, 44]. Newer assays for TSH should demonstrate negligible HCG cross-reactivity and not measure HCG [45].

A second condition, struma ovarii, constituted by the presence of thyroid follicular tissue in the ovary, may potentially present with hyperthyroidism. In such cases, suppressed TSH in combination with the absence of thyroid enlargement in the neck may provoke a question of preanalytical variables [46, 47].

**TSH-Dependent Pathophysiological Influences**

Central hypothyroidism is discussed elsewhere and is mentioned here as the "common" preanalytical disease state that is dependent on TSH secretion or the lack thereof. Inappropriate secretion of TSH in the face of clear clinical and biochemical evidence of hyperthyroidism should provoke consideration of preanalytical variables (Table 3). Growing evidence indicates that TSH-producing pituitary adenomas and genetically determined states of resistance to thyroid hormones are responsible for the phenomenon [48]. In short, the preanalytical individual variable is disease and not artifact or analytical inaccuracy. Pituitary adenomas that secrete TSH are being recognized.
Table 3. Causes of detectable TSH in hyperthyroidism.

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<th>Resistance-to-thyroid-hormone states</th>
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<td>Pituitary</td>
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<td>Pituitary TSH-secreting adenoma</td>
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<td>Ectopic secretion of TRH or TSH</td>
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<td>Artifact</td>
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<tr>
<td>Autoantibodies to TSH</td>
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<td>Human anti-mouse antibodies</td>
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earlier as a result of the newer sensitive TSH assays being used routinely [49]. Measurement of the α-subunit of TSH is highly informative [50]. Over 80% of TSH-secreting pituitary adenomas cosecrete free α-subunit [51]. This may be the sole distinction from pituitary resistance if imaging studies fail to disclose pituitary enlargement [51].

Syndromes such as "resistance to thyroid hormones" may be generalized, in which case all tissues including both pituitary and peripheral organs are resistant to thyroid hormone [52]. Selective pituitary resistance or, in some cases, with normal pituitary response, is accompanied by a peripheral organ resistance [53]. Each is recognized by the presence of an apparently inappropriate pattern of TSH and thyroid hormone secretion. The first concern of the clinician and clinical chemist is with preanalytical or analytical variables. Thus, with increased confidence in TSH and FT₄ assays and their routine application, these diagnoses are being recognized more frequently. Through the use of molecular approaches, >20 mutations have been documented [54].

Psychiatric States
Transient TSH increase and T₄ abnormalities in psychiatric patients are documented but poorly understood [4, 55]. Measured abnormalities of thyroid function should be repeatable in 2 to 4 weeks before instituting therapy in acutely hospitalized psychiatric patients. Studies correlating other findings, such as the presence of diagnostic antibodies associated with thyroiditis, assist in establishing the significance of these findings.

Smoking
Ericsson and Lindgärde [56] identified a significant effect on the thyroid gland and its function consistent with measured gland enlargement and even associated thyrotoxicosis in the predisposed individual. In pregnant smokers, enlargement of the fetal thyroid gland may be significant, particularly in geographic areas with borderline acceptable dietary iodine intake [57]. Thyroid-associated ophthalmopathy is reportedly more common and more severe in cigarette smokers [56], but was not associated with relapse following carbimazole treatment for hyperthyroidism [58].

Malabsorption or Noncompliance
Malabsorption should be considered when preanalytical assessment of variables is provoked by persistent increase of TSH after T₄ replacement therapy is initiated [18]. Although uncommon, it is clearly described in cases of patients with known short bowel as a result of surgical resection [59]. Noncompliance may be responsible and at times presents with an inappropriate increase of both T₄ and TSH resulting from an attempt to “catch up” by the hypothyroid patient taking medications in the several days before a return visit to the physician [18]. In response to T₄ administration, TSH is suppressed in a three-phase response over time [60]. Thus, normal T₄ concentrations were reported with increased TSH [18]. This may account for the seeming anomaly. Clearly, restoration of TSH into a physiological range is the accepted goal of replacement therapy [18, 61, 62].

Iatrogenic Preanalytical Influences
These are both common and significant. Prior therapy directed toward the thyroid gland, including thyroidectomy, radiotherapy to the neck, or drug therapy, would seem obvious but may be overlooked if an appropriate clinical history is not obtained. These are discussed elsewhere.

Drug Therapy
Drug therapy causes less confusion in assessing thyroid function with TSH-based strategies than was formerly encountered with measurement of TT₄, since the numerous drug-induced T₄-binding globulin variations become essentially irrelevant. Variations in T₄ therapy and compliance should not be overlooked [18, 62] with regard to other drugs that may affect thyroid testing. The major impact is experienced with inpatients, since these drugs are primarily used in a hospital setting. For this reason, some authors recommend avoidance of routine measurement of TSH and FT₄ on inpatients [4]. Through this impact on the synthesis, transport, and metabolism of thyroid hormones, or the hypothalamic–pituitary–thyroid axis, many drugs can potentially alter thyroid function and the concentrations of related hormones [63–65]. Specifically, the effects of amiodarone include not only those associated with iodine influences on the thyroid gland but also direct cytotoxic effects on thyroid epithelium. This has been shown both in vivo and in vitro in tissue culture [65]. Consequently, no simple pattern of TSH/FT₄ results can be ascribed to this preanalytical influence.

Glucocorticoids have significant impact. A single dose of dexamethasone was shown to acutely lower T₄ concentrations, altering deiodination of T₄ [66]. There are minor changes of T₄ and FT₄. Dexamethasone has been shown to diminish basal and stimulated secretion of TSH in response to TRH after administration of a 16-mg dose daily for 2.5 days [67]. Either iatrogenic exposure or (nonthyroid) illness-associated stress may produce these findings.

Abrupt lowering of the glucocorticoid concentration, as in surgical correction of Cushing syndrome or withdrawal of high-dose glucocorticoids used for immune suppression, may produce rebound thyroiditis and associated thyroid dysfunction. An alternative mechanism lies in the possible exposure of these individuals to organic and inorganic iodides in preoperative imaging studies while being evaluated for Cushing syndrome [68].

Dopamine is frequently found in association with nonthyroidal illness. Reduction of TSH secretion in euthyroid patients as well as in hypothyroid patients establishes this agent as a
significant potential confounding preanalytical variable [69, 70]. As a consequence, confident assessment of true thyroid-functional status may be precluded in these patients. The administration of dopamine induces acute reduction of TSH secretion, which rebounds promptly when dopamine is discontinued. So significant are these changes that it has been cited as inducing iatrogenic hypothyroidism [71].

Lithium is widely used in psychiatric disorders [72]. Increase in TSH occurs in 10–20% of treated patients and results from inhibition of T4 synthesis and release. An increase in thyroid antibodies has been observed [72]. In lithium-treated patients, pituitary secretion of TSH appropriately responds to T4 supplementation therapy [72].

Anticonvulsant drugs, particularly phenytoin and carbamazepine, affect not only T4 binding but also thyroid hormone metabolism and TSH secretion, but TSH is not increased in basal samples. T4 and FT4 are decreased 25–30% [63, 73]. As such, a variety of changes are seen, usually including reduction of TT4 and FT4. Bromide exposure has also been linked to impaired thyroid function [74–76]. Sangster et al. failed to demonstrate an effect of bromide on man in a careful dosing study [74]. In vitro studies and studies in rats clearly show the potential for a bromide effect [75]. Allain et al. cogently summarize the evidence, concluding that bromide is unlikely to commonly interfere with assessment of thyroid function [76].

Povidone–iodine skin cleaning preparations used in hemodialysis, peritoneal dialysis, treatment of decubitus ulcers, and other conditions may lead to hyperthyroidism and hypothyroidism by altering TSH and FT4 concentrations in those who are predisposed. Easily overlooked, this cause should be considered in assessment of possible preanalytical states [77, 78].

Plasmapheresis with plasma exchange has been shown to acutely lower thyroid-binding proteins and associated concentrations of TT4 and TT3, possibly in association with diminished peripheral deiodination of T4 [79].

**Specimen-Based Influences**

As a result of TSH-based strategies and improved assays for TSH and FT4, specimen-related influences on thyroid-function testing are less frequently significant. Improved precision and reduction of these influences permits an approach to published goals of hormone testing [80, 81]. However, these considerations depend not only on the degree of intraindividual variation and the impact of the interference, but also on the relative magnitude of these compared with observed population reference values. When applied serially for an individual, the more subtle specimen influences become important as a result of the set-point precision associated with the individual's FT4 concentration and the log-linear TSH response to small FT3 deviations within the reference range. Kroll and Elin [9] provide a recent update, noting the method-specific nature of interference, particularly with immunoassays incorporating a washout step. They emphasize the importance of manufacturer sources of specific information, notably the package insert for reagents. This is particularly true for automated methods. Generally, hemolysis, lipemia, and icterus are less significant for immunoassays [82, 83] or competitive-binding assays [84] than for classic colorimetric analytical methods [85].

Stasis from tourniquet application continues to be important in regard to specimen collection, especially for peptides and proteins that do not equilibrate with the interstitium. Proteins increase ~3–5% with long tourniquet times [86].

T4 stability is good when stored at 4 °C or frozen at −20 °C for months, as noted in earlier publications [87]. The possibility of protein precipitation has been noted and affected earlier assays. More recently, gel barrier tubes have been reported to have no adverse effect on TT4 concentrations, although progesterone was significantly affected [88].

An extensive study compared collection systems and storage at room temperature or at 4–8 °C. This included serum in plain glass tubes or barrier gel, and plasma collected in either EDTA or heparin (Table 4) [89]. These investigators measured the effects on one assay for TSH and two for FT4. Specimens were stored 1 h–13 days. Also studied were the effects on whole blood of storage for 1 h–5 days. In summary, a variety of changes were observed for all collection and storage conditions; however, serum drawn in glass and stored at 4–8 °C was least affected. TSH increased gradually when stored at room temperature. Generally, changes were slight and not likely to alter clinical categorization. Literature reports indicate serum as the preferred specimen for measurement of FT4 concentrations obtained from prompt centrifugation of whole blood, and storage of the serum at −20 °C if the assay is to be delayed [90, 91]. Others, investigating complex variables regarding FT4 assays, are notably silent with regard to stability and collection issues [92, 93]. Similarly, studies regarding TSH make little mention of stability, collection, or storage [94–97]. Studies on serum TSH indicated no stability problem with an earlier assay in conjunction with a complex chromatographic extraction of TSH [98]. Expected recovery was 76.6%, but these studies are probably not comparable with routine clinical application. Recently, extensive data have been published repeating the observation of Nishi et al. [89], that TSH may increase slightly when stored or transported at ambient temperature for long periods [99]. The changes were seen with some but not all sample pools tested and are a subject of debate [100, 101]. Studies of TSH in protein-free buffer revealed dissociation of TSH into its subunits at −20 °C but not at 4 °C or room temperature [102]. These workers reported agreement with earlier reports of satisfactory stability of TSH in serum. One notable exception occurred in a report regarding collection of serum for TSH assay in silicone-coated microcontainer collection tubes (Vacutainer Tube; Becton Dickinson, Rutherford, NJ) and one specific assay. Wickus et al. described a negative effect of silicone on the biotin–avidin system used in this assay [103]. This phenomenon illustrates the importance of assay-specific variables.

Dried whole-blood spots have been validated widely for stability of use in neonatal screening programs [104] for congenital hypothyroidism. TSH and TT3 are more stable than TT4 in dried blood under various storage conditions [105]. Assay of FT4 has been successfully applied to dried blood spots [106].

Lipemia contributes preanalytical error to immunoassay for
thyroid testing [107, 108], primarily as a result of partitioning and solute exclusion as in pseudohypokalemia, resulting in underestimation of the analyte. This is unlikely to significantly affect assessment of thyroid function. However, free fatty acids have long been associated with interference in T₄ assays. RIAs are less affected than competitive protein-binding methods [109]. More significantly, free fatty acids are well known to perturb assays for FT₄ by displacement of T₄ from binding proteins [110]. This explains, in part, the confusing values seen in nonthyroidal illnesses [110, 111], and additionally reinforces reliance on the TSH assay in preference to FT₄ [112].

Variable protein binding associated with familial dysalbuminemia, as well as serum albumin fluctuations, significantly compromise assays for FT₄ measurements [2, 113, 114].

Paraproteinemia causes interference in many assays. At times, the viscosity may produce a “short” sample, resulting in falsely low values. At other times, paraproteins nonspecifically bind either analytes or reagents, which may variably affect the result [9].

Background specimen contamination introduced as a preanalytical activity may also affect the assay, as in the case of intravenous fluorophore use for ophthalmic procedures [115, 116]. This interferes with the fluorescent signal in a specific method (TT₄; Abbott Diagnostics, Chicago, IL). Similarly, the problem of background radioisotope contamination affects dialsates for FT₄ assay as an occasional problem for those still using isotope immunoassays [117].

Antibodies may be helpful; e.g., antithyroid peroxidase in thyroiditis provides corroboration of thyroid disease [118, 119]. In a few cases, autoantibodies to T₃ and T₄ develop, which increase the circulating concentrations of TT₃ or TT₄, or both [120, 121]. Sensitive testing screened 880 apparently well adults and found an incidence of anti-T₃ or anti-T₄ as high as 1.8% [122]. The report indicates minimal effect on the assays in these individuals. Recently, anti-T₃ autoantibodies were reported to interfere with immobilized T₃ in a solid-phase assay for FT₄, resulting in spuriously high FT₄ measured values. Once again, the unique susceptibility of assays to individual preanalytical variables is demonstrated [123] and also is seen with other FT₄ methods [124].

Antibodies to TSH were originally described in Graves disease and may alter assay results for this hormone [125–127], but are believed to be unlikely to activate the TSH receptor. Since TSH is now measured more often, spuriously TSH increase may be recognized more frequently.

Paraproteinemia, typically human anti-mouse antibodies, are encountered with moderate frequency when using assay kits with mouse monoclonal reagents and antibodies [128]. These may be nonspecific but can significantly alter results [129]. Although manufacturers add mouse serum to reagent systems to neutralize these antibodies, it may be insufficient in some cases to avoid confounding results [130–132]. Autoantibodies to TSH and other pituitary hormones are more common in the empty sella syndrome and with pituitary tumors [133]. When thyroglobulin is assayed, anti-thyroglobulin antibodies should routinely be sought since they are significant, common, and may invalidate the assay [134].

HUMAN ERROR

A final category representing an inevitable preanalytical error includes errors on order entry [135], phlebotomy error, mislabeling, erroneous aliquots, sequencing errors, barcode errors, and the like [136]. With modern systems, this can be reduced, as recently reported [137], but it would be foolish to reject consideration of this variable. For the individual patient, one event represents 100%. The occasional error nurtures the clinical aphorism that laboratory data should be rejected if not in keeping with clinical judgment. This is hardly appropriate, now that subclinical hypothyroidism [18] and subclinical hyperthyroidism [138] both depend on TSH assay data in the absence of clinical findings. Error is far more common in medicine than we would like to believe, reportedly affecting >20% of patients admitted to a university hospital [139]. Study of error may be highly beneficial. One should approach human error in medicine as “medical treasures,” sometimes called “gems” [140]. They present opportunities to learn: to improve practices and to introduce systems for future avoidance of recurrences [140].

Table 4. Summary of the effect of various sampling and storage conditions on TSH and FT₄ values.

<table>
<thead>
<tr>
<th></th>
<th>Kit</th>
<th>Temp.</th>
<th>Glass</th>
<th>Separator</th>
<th>EDTA-2Na</th>
<th>Heparin-Na</th>
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<tr>
<td>Blood kept in tubes from 1 h to 5 days</td>
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<td>Amerlex-M Free T₄</td>
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<td>Serum or plasma stored from 1 h to 13 days</td>
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<td>SPAC-S TSH</td>
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</table>
→, no change; ↗, decrease; ↘, increase.

*4–8 °C.

References


48. Weintrub BD, Gershengom MG, Kourides IA, Fein H. Inappropria-


89. Midgley JE, Sheehan CP, Christofides ND, Fry JE, Browning D, Mardell R. Concentrations of free thyroxin and albumin in serum
120. Ginsberg J, Segal D, Ehrlich RM, Walfish PG. Inappropriate triiodothyronine (T3) and thyroxine (T4) radioimmunoassay levels secondary to circulating thyroid hormone antibodies. Clin Endocrinol 1978;8:133–9.


