EnM OPEN ACCESS
ENDOCRINOLOGY AND METABOLISM

Editorial

Endocrinol Metab 2025;40:866-868
https://doi.org/10.3803/EnM.2025.2822
pISSN 2093-596X · eISSN 2093-5978

# LC-MS/MS for Thyroglobulin: A Complementary Approach to Immunoassay Limitations for Thyroid Cancer

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Thyroglobulin (Tg) is an important tumor marker for monitoring recurrence in patients with differentiated thyroid cancer (DTC). Guidelines emphasize using serum Tg levels and neck ultrasound to detect persistent or recurrent disease during follow-up. Rising Tg can signal residual or recurrent disease, making sensitive and specific Tg assays essential. However, a major challenge in conventional Tg immunoassays is interference from anti-thyroglobulin autoantibodies (TgAb), which are present in up to 25%–30% of patients with DTC [1].

Immunometric assays, including the immunoradiometric assay (IRMA) and chemiluminescence microparticle immunoassay (CMIA), are widely used to measure Tg levels because of their high sensitivity and automation. However, these assays are prone to interference from TgAb, which can bind to Tg and cause falsely low or undetectable Tg levels. These autoantibodies can bind to circulating Tg and interfere with sandwich immunoassays by masking antibody epitopes on Tg. As a result, serum Tg can be underestimated in TgAb-positive patients, even when residual cancer is present. The degree of interference can vary with TgAb titer and epitope specificity, making it difficult to predict the magnitude of assay distortion in a given patient [2]. In some cases, TgAb or other heterophile antibodies can artificially increase serum Tg levels, but false low results remain more common [3].

In this context, when a patient is TgAb-positive, clinicians cannot fully trust a low or undetectable Tg result from immunoassays. In practice, physicians must instead rely on alternative indicators of recurrence, such as neck ultrasound findings or trends in TgAb levels over time. Notably, rising TgAb titers can function as a surrogate tumor marker, as a persistent increase is associated with recurrent or residual disease in patients whose Tg appears falsely negative. However, tracking TgAb trends is an indirect and delayed approach. This ongoing clinical dilemma underscores the need for a method that can accurately quantify Tg even in the presence of antibodies. This persistent challenge highlights the urgent need for a reliable technique capable of accurately measuring Tg levels despite interfering antibodies.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) offers a fundamentally different approach to Tg measurement and overcomes the interference issues seen with immunoassays. Instead of relying on antibody–antigen binding, the MS-based assay detects Tg-derived peptide fragments by their mass-to-charge signatures. In a typical workflow, serum proteins are enzymatically digested to release Tg-specific peptides, which are then quantified by the mass spectrometer. This method avoids the problem of TgAb masking epitopes on the full Tg protein because it detects Tg based on peptides generated regardless of any antibody binding in the original sample. Studies have previously confirmed that LC-MS/MS can accurately measure Tg even when autoantibodies are present. Kushnir et

**Received:** 27 November 2025, **Accepted:** 8 December 2025

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al. [4] demonstrated that a mass spectrometry assay recovered true Tg concentrations in TgAb-positive sera, whereas conventional immunoassays produced falsely low values.

LC-MS/MS has shown advantages across endocrine assays in which immunoassays often fail. Low concentrations of steroid hormones, including testosterone and cortisol, are frequently susceptible to cross-reactivity or drug interference, which LC-MS/MS can address through superior analytical specificity [5]. It also accurately distinguishes 25(OH)$D_2$ and $D_3$, avoiding the underestimation commonly observed in vitamin D immunoassays, and provides interference-free insulin-like growth factor 1 measurements by eliminating the effects of binding proteins. Together, these examples demonstrate how mass spectrometry enhances accuracy and reliability across endocrine diagnostics [6,7].

In the recent study by Park et al. [8], the LC-MS/MS method successfully detected Tg in samples where conventional immunoassays failed because of the presence of TgAb. It was found that 76.2% of patients with thyroid carcinoma had TgAb levels exceeding 60 U/mL. This high prevalence underscores the clinical importance of addressing the limitations of current assays. While IRMA and CMIA correlate well in TgAb-negative patients, they perform poorly in those who are TgAb-positive, as reflected by reduced correlation coefficients. Moreover, the study showed that in the presence of TgAb, conventional assays often failed to detect Tg, whereas LC-MS/MS identified some of these missed cases, indicating its potential value as a complementary method in selected clinical situations.

Although LC-MS/MS overcomes an important limitation of immunoassays, it presents its own challenges that must be considered. Most clinical Tg immunoassays achieve very low detection limits (often <0.1 ng/mL), whereas current LC-MS/MS assays typically have detection thresholds on the order of 0.5–1.0 ng/mL, which could miss extremely low Tg levels [9]. Improving the sensitivity of MS-based Tg tests, therefore, remains a priority. In addition, LC-MS/MS requires expensive instrumentation and specialized expertise, which limits its availability to certain laboratories, whereas immunoassays are faster, more cost-effective, and well suited for high-volume thyroid cancer monitoring. Finally, the long-term impact of LC-MS/MS on patient outcomes is still uncertain. Although it can detect Tg that immunoassays miss, it remains unclear whether this translates into improved recurrence detection or survival. More evidence is needed to evaluate its benefits relative to existing strategies.

Given the strengths and limitations of both methods, LC-MS/MS should complement Tg immunoassays rather than replace them. Immunoassays remain the primary tool for patients without TgAb because of their high sensitivity and automation. However, LC-MS/MS is valuable in TgAb-positive patients with undetectable or low Tg by immunoassay, helping uncover hidden Tg and prompting further evaluation [10]. It is also useful when clinical evidence of recurrence does not match low Tg levels obtained from immunoassays. Monitoring TgAb trends remains important, with significant rises indicating the need for further assessment.

Persistent interference in Tg immunoassays, particularly from anti-TgAb, has complicated thyroid cancer monitoring. LC-MS/MS provides a complementary solution to these limitations. While further research will help clarify its clinical role, incorporating LC-MS/MS for TgAb-positive patients may represent a significant advance in improving thyroid cancer management.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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