Letters to the Editor

denaturation times can be controlled to 1-s accuracy. Although our testing was limited to fast-COLD-PCR, we anticipate that the current approach may also be applicable to other COLD-PCR formats, including full-COLD-PCR (6), ICE (improved and complete enrichment) COLD-PCR (7), and temperature-tolerant COLD-PCR (8).

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References


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Usefulness of a Thyroglobulin Liquid Chromatography–Tandem Mass Spectrometry Assay for Evaluation of Suspected Heterophile Interference

To the Editor:

The accuracy of human serum thyroglobulin (Tg)1 measurement by immunoassays can be affected by anti-Tg autoantibodies (TgAB) (1). In immunometric Tg assays, which dominate the market, TgAB interference can result in false-low measurements. Tg quantification by liquid chromatography–tandem mass spectrometry (LC-MS/MS) was first described by Hoofnagle et al. (2) and subsequently shown by Clarke et al. to overcome TgAB interference by the use of proteotypic peptide quantitation after tryptic digestion, with peptide-specific immunocapture (3). In the June 2013 issue of Clinical Chemistry, Kushnir et al. presented a study that further solidified the usefulness of using LC-MS/MS in overcoming the effects of TgAB on accurate Tg quantification by demonstrating that 23% of TgAB-positive serum samples, which had undetectable Tg concentrations by immunoassay (<0.1 μg/L), had Tg values above the limit of quantification (LOQ) of their LC-MS/MS Tg assay ≥0.5 μg/L (4).

We read this study with great interest because we recently implemented a similar methodology in our laboratory. In TgAB-positive/ Tg-negative samples by immunoassay, our results matched the findings of Kushnir et al. very well (21% positive by LC-MS/MS, n = 115). Given the success in overcoming TgAB interferences, we believed that this methodology would overcome interferences due to heterophilic antibodies (HAB), which may result in false-high Tg measurements in immunometric assays. The current process of workup of samples with suspected HAB is complex and costly. It involves remeasurement of the questionable sample after serial dilu-

1 Nonstandard abbreviations: Tg, human serum thyroglobulin; TgAB, anti-Tg autoantibodies; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LOQ, limit of quantification; HAB, heterophilic antibodies.
tions, pretreatment with HAB blocking reagents, and reassaying with alternative immunoassay platforms. While this workup usually succeeds in confirming the presence of HAB interference, it frequently fails to provide an accurate result of the true Tg concentration. The goal of this study was to determine whether Tg measurement by LC-MS/MS would allow accurate quantification in samples with suspected HAB interference.

This study was determined to be exempt by the Mayo Clinic institutional review board. During a 4-month period, our laboratory received requests for investigation of potential HAB interference in the Tg immunoassay (Access, Beckman Coulter) results for 7 patients. HAB interference was excluded when (a) serial dilutions of the sample on the Access Tg assay were linear (recovery of dilutions 100–15% of the original value), (b) pretreatment with HAB blocking reagents did not substantially alter the results (<20% difference between treated and untreated samples), and (c) retesting with a different Tg assay (Immulite, Siemens Corporation) yielded similarly increased values. In all other cases HAB interference was considered likely.

Each sample was then assayed for Tg using LC-MS/MS. Briefly, large proteins, including Tg, were selectively precipitated with ammonium sulfate. Pellets were resuspended in ammonium bicarbonate buffer (pH = 8) and reduced and alkylated with dithiothreitol and iodoacetamide, respectively. C13-labeled stable isotope internal standard peptide was added, and the samples were digested with bovine trypsin for 16 h. Antibody-labeled magnetic beads were then added to capture the proteotypic Tg peptide of interest (FSPDDSAGASALLR), which was assayed postelution by a single-reaction monitoring–based MS/MS method on an API 5000 (AB SCIEX) mass spectrometer operating in positive electrospray ionization mode. Three ion transitions each were monitored for the native peptide and its corresponding internal standard.

Results of the Access Tg and LC-MS/MS Tg matched for the 4 samples with negative HAB workup (Table 1). The other 3, likely HAB-positive samples, had serum Tg concentrations below the LOQ (<0.5 μg/L) by LC-MS/MS, but measured as high as 604 μg/L on the Access. In all 3 cases the clinical presentation was concordant with the absence of disease, as predicted by the LC-MS/MS assay results. In 2 of these cases, the traditional HAB workup had only ascertained the presence of HAB interference but had failed to provide a reliable quantitative Tg result. In these cases, pretreatment with heterophilic blocking reagent had not reduced the apparent Access Tg concentration to a value comparable to the corresponding LC-MS/MS measurement, and these samples also showed increased Tg concentrations on the Immulite platform, albeit lower than by the Beckman assay.

We conclude that the determination of Tg concentrations by LC-MS/MS obviates the need for extensive HAB workup by classical methods, while providing accurate quantification of Tg in the presence of substantial HAB interferences. We propose adopting this approach for all cases of suspected HAB interference in Tg measurement, and hypothesize that this approach could be extended to other analytical targets that can be measured by LC-MS.

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