Evaluation of two LC-MS/MS thyroglobulin assays performance in the presence of anti-thyroglobulin autoantibodies

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Introduction: Measurement of thyroglobulin (Tg) by LC-MS/MS (Tg-MS) has recently been introduced in the clinical laboratories as a mean to obtain accurate Tg quantitation in the presence of anti-Tg autoantibodies (TgAB), a limitation in current immunoassays. Standardization between Tg-MS assays is paramount to the success of clinical adaptation and their use in the long term follow-up of thyroid cancer patients. The goal of this study was to compare the performance of two Tg-MS methods in a well characterized set of TgAB positive and negative serum samples and contrast with the performance of both an immunometric assay and radioimmunoassay under the same specimen conditions.

Methods: Standardized Tg mixtures (1, 25, 100 ng/mL) were created by spiking a serum pool negative for both Tg and TgAB, with Certified Reference Material (CRM-457). A polyclonal TgAB mixture, that was generated from serum samples containing high levels of TgAB but with undetectable Tg, was spiked into each Tg concentration to obtain five concentrations of TgAB from 10 to 2500 IU/mL. For method comparison studies, serum samples from patients with a diagnosis of thyroid cancer were evaluated. Samples were classified as Tg and TgAB positive or negative by the Beckman DXI and Roche Elecsys assays, respectively. Standardized mixtures and serum specimens were run by Tg-MS (Mayo Clinic and University of Washington), Beckman Access Tg assay (per manufacturer's instruction), and a radioimmunoassay (Queen Elizabeth Hospital, Birmingham, UK).

Results: Fold-change analysis for standardized mixtures varied between assay types: Tg-MS assays varied minimally (-0.2 to +0.2-fold) regardless of antibody concentration. Underestimation was observed in the Beckman Tg assay, -0.3 to -7.0-fold as antibody concentration increased. In the RIA, overestimation of +0.3 to +10.7-fold was observed, with higher fold-increases observed in preparations containing lower Tg concentrations. Regression

analysis between the two Tg-MS assays for all samples (N = 115) showed good correlation (Slope = 1.1; R^2 = 0.97, intercept <0.5). Concordance analysis for TgAB negative (N = 48) and TgAB positive (N =67) between the two Tg-MS assays showed three discrepant specimens which were Tg positive by Mayo's method, and Tg negative by the University of Washington's. Two of three specimens were at or near the Mayo assay's LOQ (0.5 ng/mL).

Conclusion: In contrast to the immunoassays, the two Tg-MS methods evaluated were not susceptible to TgAB interference even at the high TgAB concentrations tested. The excellent assay correlation and concordance (97%) between the two assays highlights the robustness of the methods for use in a clinical setting. Broader ranging comparative studies are warranted between sites performing Tg-MS to further substantiate clinical adaptation and long-term monitoring usefulness.