Nonstandard abbreviations: TgAb, antithyroglobulin

To the Editor:

Antithyroglobulin autoantibodies (TgAbs) frequently cause falsely low measurements of serum thyroglobulin (Tg) when immunometric assays (TgIAs) are used (1). This problem can be overcome by tryptic digestion of patient serum with subsequent measurement of Tg proteotypic peptides by LC-MS/MS (TgMS) (2-4). However, it remains uncertain how different TgMS assays compare with each other, a crucial question in today’s laboratory testing environment, wherein concern has been raised over patient samples being tested by different methods or in different laboratories over time (5). We therefore performed a pilot study to assess the intermethod concordance between 4 independently developed TgMS methods. The study was approved by the Mayo Clinic institutional review board.

We tested 40 patient serum samples spanning Tg concentrations from <0.1 to 10 ng/mL (assigned by DxI TgIA, Beckman Coulter). We classified the samples as TgAb positive (n = 22) or TgAb negative (n = 18) on the basis of whether they contained TgAb concentrations that exceeded the functional sensitivity of the DxI TgAb assays (1.8 IU/mL). Samples were deidentified and shared between 4 laboratories for TgMS testing: ARUP Laboratories, Laboratory Corporation of America Holdings (LabCorp), Mayo, and the University of Washington (UWash). Each assay uses different sample preparation methodologies: ARUP and Mayo use different protein precipitation steps to enrich for thyroglobulin before proteolysis, and LabCorp and UWASH digest serum samples directly after different denaturing steps. The peptide targets are also different between the assays: ARUP enriches and monitors the peptide VIFDANAPVAVR, whereas LabCorp, Mayo, and UWASH enrich and monitor the peptide FSPDSSAGASALLR for primary quantification.

Mayo calibrators were also shared between laboratories (Tg-negative/TgAb-negative pooled serum spiked with international reference material BCR-457 and run as unknowns in each assay. A different batch of samples (because of sample volume limitations), also spanning Tg concentrations <0.1–10 ng/mL and with similar TgAb status, was assayed across 4 automated TgIA assays: DxI-Tg, Immulite®-Tg (Siemens Corp.), Elecsys® Tg II (Roche Diagnostics), and Kryptor-Tg (Thermo Fisher).

Comparison of Mayo calibration material between TgMS methods showed excellent correlation but substantial differences in calibrator assignment. With Mayo values as a reference, slopes were 0.826, 0.878, and 0.814 for ARUP, LabCorp, and UWash, respectively, and r² values were 0.999, 0.999, and 0.994. The variation in the observed slopes is likely explained by the different approach to calibration: ARUP and LabCorp use the calibration materials provided with the DxI assay, Mayo uses BCR-457 spiked into Tg-negative serum, and UWash uses human samples assigned by the DxI assay.

Comparison of TgMS methods in the patient samples also showed good correlation (Fig. 1). Defining the mean Tg value (in nanograms per milliliter) across all 4 TgMS assays as the reference, slopes across patient samples were 0.833, 1.027, 1.043, and 1.102 for ARUP, LabCorp, UWash, and Mayo, respectively, and r² values were 0.961, 0.996, 0.981, and 0.988. When the Mayo assay’s patient values and calibrators were used as the reference instead, slopes were 0.740, 0.920, and 0.942 for ARUP, LabCorp, and UWash, respectively, and r² values were 0.932, 0.983, and 0.970. The contribution of the calibration bias to the slope could be seen when the values were adjusted for each assay’s calibration bias vs the Mayo calibrators. This yielded slopes of 0.895, 1.05, and 1.16 and identical r² val-
ues as above for ARUP, LabCorp, and UWash, respectively.

These comparative linear-fit data for the TgMS assays were similar to those obtained when comparing the different TgIAs with each other. The slopes of the TgIAs to the all-TgIA mean were 1.029, 0.876, 0.803, and 1.297 for DxI, Immulite, Kryptor, and Elecsys TgII, respectively, and \( r^2 \) values were 0.983, 0.911, 0.971, and 0.993. Judging by the agreement in the slopes (relative SD 11.6% for TgMS vs 21.9% for TgIA) and the more favorable \( r^2 \) values (mean 0.98 for TgMS vs 0.96 for TgIA), the harmonization between the MS-based assays is at least as good as, or probably better than, the harmonization between the TgIAs. This is remarkable given the differences in target peptide, sample preparation, choice of isotopically labeled internal standards, and calibration across the 4 TgMS assays.

It is important to point out that the monoclonal antibody to the peptide FSPDDSAGASALLR was developed by the Clinical Proteomic Technologies for Cancer Initiative, a multicenter consortium funded by the National Cancer Institute from 2006 to 2011. Its direct application in the care of patients is a great advance for laboratory medicine. An expanded repertoire of antipeptide antibodies would help facilitate better patient care in other important disease areas.

The mass spectrometric measurement of Tg is resistant to the effects of autoantibodies. Our small pilot study further demonstrates that even with extensive sample preparation, multiple laboratories can achieve similar results to one another. However, additional steps toward harmonization of Tg assays will surely benefit TgMS assays as much as they would immunoassays in helping to ensure excellent comparability of results across assays in many laboratories.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the
article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: R.P. Grant, Laboratory Corporation of America; C.M. Shuford, Laboratory Corporation of America Holdings.

Consultant or Advisory Role: None declared.

Stock Ownership: R.P. Grant, Laboratory Corporation of America.

Honoraria: None declared.

Research Funding: NIH grant CA160034-04.

Expert Testimony: None declared.

Patents: A.N. Hoofnagle, patent 7,807,172.

References

1. Spencer CA. Challenges of serum thyroglobulin (Tg) measurement in the presence of Tg autoantibodies. J Clin Endocrinol Metab 2004;89:3702–4.


5. Spencer C, Petrovic I, Fatemi S, LuPresti J. Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer with sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. J Clin Endocrinol Metab 2014;99:4589–99.

Brian C. Netzel2
Russell P. Grant3
Andrew N. Hoofnagle4,5
Alan L. Rockwood4,7
Christopher M. Shuford3
Stefan K.G. Grebe2,8*

2 Department of Laboratory Medicine and Pathology
3 Laboratory Corporation of America
4 Burlington, NC

* Address correspondence to this author at:
Hilton 730C
Mayo Clinic
200 1st St SW
Rochester, MN 55905
Fax 507-284-9758
E-mail grebe.stefan@mayo.edu

Previously published online at DOI: 10.1373/clinchem.2015.245266