Supplementary Data

Supplementary Method

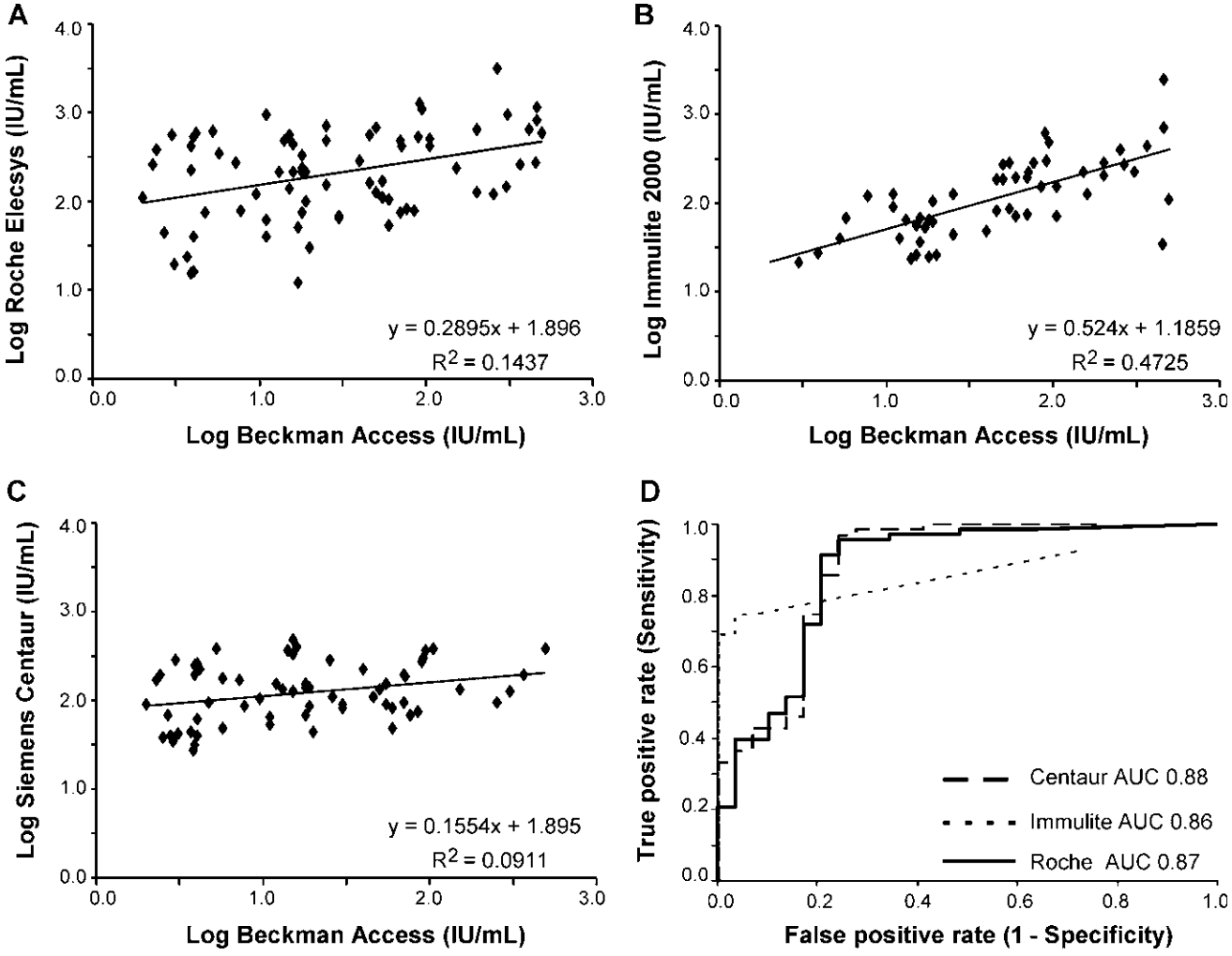
This study was approved by the Mayo Clinic Institutional Review Board.

Assays

All thyroglobulin (Tg) measurements were performed using the Beckman-Coulter Access Tg assay (which remains available).

Four FDA-cleared thyroglobulin autoantibody (TgAB) assays were evaluated: Access TgAB on the UniCel DxI 800 (Beckman-Coulter), Immulite 2000 anti-TG AB (Siemens), Advia Centaur anti-Tg (Siemens), and Elecsys anti-Tg assay on the Cobas e601 (Roche Diagnostics). The lowest reportable concentrations of these assays are 1.8 IU/mL (Beckman-Coulter Access), 20 IU/mL (Siemens-Immulite 2000), and 10 IU/mL (Siemens-Centaur and Roche, respectively).

We have used the Beckman-Coulter Access TgAB assay to compliment thyroid cancer Tg testing since 2004, employing a thyroid cancer–focused cutoff of 4 IU/mL for TgAB positivity, rather than the much higher cutoff used for thyroid autoimmunity in the absence of thyroid cancer. The 4 IU/mL cutoff is



SUPPLEMENTARY FIG. S1. Method comparison of TgAB assays. Relationship of log-transformed TgAB (log10, IU/mL) data of the Roche Elecsys **(A)**, Siemens Immulite 2000 **(B)**, and Siemens Advia Centaur **(C)** to the Beckman-Coulter Access assay. The equation for the linear fit of the log-transformed data and the r^2 are shown. The log-transformed data did show a weak-to-modest linear relationship, but large intercepts were present and slopes ranged from 0.15 to 0.52 with r^2 of only 0.09–0.47. **(D)** Receiver-operating characteristics curve analysis for the Roche Elecsys, Siemens Immulite 2000, and Siemens Advia Centaur immunoassays. The results of the Beckman-Coulter Access TgAB assay with a cutoff of 4 IU/mL were used as the reference method to categorize samples as TgAB positive or negative. TgAB, thyroglobulin autoantibody.

Supplementary Table S1. Thyroglobulin Autoantibody Immunoassays Concordance in the Clinical Validation Sample Group

	Beckman-Coulter DxI Negative (<4.0 IU/mL)	*Beckman-Coulter DxI Positive (≥4.0 IU/mL)*	*Kappa (95% CI)*
Roche Elecsys			
Negative (<22 IU/mL)	51	3	0.94
Positive (≥22 IU/mL)	1	75	(0.87–1.00)
Siemens Immulite 2000			
Negative (<20 IU/mL)	52	28	0.59
Positive (≥20 IU/mL)	0	50	(0.47–0.71)
Siemens Centaur			
Negative (<44 IU/mL)	50	7	0.86
Positive (≥44 IU/mL)	2	71	(0.77–0.95)

CI, confidence interval.

slightly above the functional sensitivity of 1.8 IU/mL, because we have observed some false-positive TgAB results between 1.8 and 4 IU/mL, and clinically relevant interferences with Tg measurements have been rare in this low-positive range—an observation supported by the literature (1,2).

Initial method comparison and establishment of cutoffs for thyroid cancer follow-up

One hundred cryopreserved patient samples, previously tested for Tg and TgAB on the UniCel DxI 800, were used for initial method comparison. The samples spanned a range of TgAB concentrations: 15 with TgAB < 1.8 IU/mL, 17 with TgAB 1.8–4 IU/mL, 8 with TgAB 4–10 IU/mL, 44 with TgAB 10–100 IU/mL, and 16 with TgAB >100 IU/mL. The original Beckman-Coulter TgAB results were plotted against those obtained after retesting with the Roche Elecsys, Siemens-Centaur, and Siemens-Immulite TgAB assays. Receiver-operating characteristics (ROC) curves were constructed (Analyze-it® for MS-Excel) using the Beckman-Coulter TgAB assay with the 4 IU/mL cutoff as the benchmark for positivity, and optimal cutoffs were determined for the other assays.

Clinical validation

The cutoffs established by ROC curve analysis were validated in a separate set of 130 samples from 113 Mayo Clinic patients, for which complete clinical data were available. The samples were selected to include patients with (43 patients with 51 samples) and without (70 patients with 79 samples) definitive persistent/recurrent disease (clinical or imaging proven). In addition, samples were again selected to cover a range of TgAB values: <1.8 IU/mL ($N = 52$), 1.8–4 IU/mL ($N = 0$), 4–10 IU/mL ($N = 13$), 10–100 IU/mL ($N = 39$), and >100 IU/mL ($N = 26$). Categorical agreements between the four assays were tabulated, using the cutoffs established above, and kappa values for agreement were calculated. For each assay, we also tabulated the number of clinically relevant false-low/negative Tg results that were associated with TgAB positivity. A false-low/negative Tg result was defined as a Tg below either of two commonly accepted cutoffs (more recent: <0.1 ng/mL; older: <2 ng/mL) for the absence/presence of possible persistent/recurrent disease (3).

Supplementary References

1. Rosário PWS, Maia FFR, Fagundes TA, Vasconcelos FP, Cardoso LD, Purisch S 2004 Antithyroglobulin antibodies in patients with differentiated thyroid carcinoma: methods of detection, interference with serum thyroglobulin measurement and clinical significance. Arq Bras Endocrinol Metabol **48:**487–492.
2. Gorges R, Maniecki M, Jentzen W, Sheu SN, Mann K, Bockisch A, Janssen OE 2005 Development and clinical impact of thyroglobulin antibodies in patients with differentiated thyroid carcinoma during the first 3 years after thyroidectomy. Eur J Endocrinol **153:**49–55.
3. Grebe SKG 2009 Diagnosis and management of thyroid carcinoma: a focus on serum thyroglobulin. Expert Rev Endocrinol Metab **4:**25–43.