Improved Sensitivity of a Thyrotropin Receptor Antibody Assay, Esther Jensen, Per Hyltoft Petersen, Ole Blaabjerg, Pia Skov Hansen, and Laszlo Hegedüs (Departments of Clinical Biochemistry and Endocrinology and Metabolism, Odense University Hospital, Odense, Denmark; NOKLUS, Norwegian Quality Improvement of Primary Care Laboratories, Division for General Practice, University of Bergen, Bergen, Norway; address correspondence to this author at: Department of Clinical Biochemistry, Odense University Hospital, DK-5000 Odense C, Denmark; fax 45-65-41-19-11, e-mail esther.jensen@ouh.fyns-amt.dk)

The National Academy of Clinical Biochemistry guidelines recommend use of serum thyrotropin (TSH) receptor antibody (TRAb) analysis to distinguish between Graves disease and other thyroid diseases, such as subacute or postpartum thyroiditis and toxic nodular goiter (1, 2). The presence of TRAb, however, is not yet a prerequisite for the diagnosis of Graves disease.

A research laboratory has determined a functional assay sensitivity, defined as the lowest concentration with an interassay CV of 20%, for TRAb of 0.75 IU/L (3). This value was determined under optimized and standardized conditions, however, and assay performance may not be as good in other laboratories (4–7). The sensitivity of the method has, until now, been poor, and upper reference limits of 1–2 IU/L (which are probably too high) have been published. The aim of our study, therefore, was to improve the functional sensitivity of a commercial TRAb assay (Brahms Diagnostica) calibrated against WHO Standard 90/672 (3, 8).

According to the manufacturer's instructions, we added 200 μL of buffer in duplicate to tubes coated with human recombinant TSH receptor and then added 100 μL of sample/standard. The solution was incubated for 2 h at room temperature with shaking. The tubes were washed twice with 2 mL of washing solution, after which 200 μL of 125I-labeled TSH was added. After 1 h of incubation with shaking at room temperature, the tubes were washed 3 times with 2 mL of washing solution, and the radioactivity was measured for 1 min in a WIZARD 1470 automatic gamma counter equipped with a Multicalc data management program (Perkin-Elmer). The manufacturer recommends manual data evaluation by plotting B/B0 (%) on lin-log graph paper. No special computer-assisted technique is recommended.

In a pilot study, we compared 2 RIA curve-fitting methods: a model-based weighted 4-parameter logistic (4PL) method and a data-based logit-spline method (9). The tubes from a complete calibration curve and 22 randomly chosen patient samples in duplicate were counted 10 times for 1 min and 10 times for 5 min in each model, a total of 22 × 2 × 4 data sets of 10 countings. Forty-two countings were outside the calibration curve (mainly for the 4PL model), but a total of 134 had all 10 results for estimation of mean and CV. The curve-fitting quality in the lower end of the calibration curve can be judged from the variation of the estimated concentration, e.g., 80% bound (ED80) for the 4 combinations: 4PL (60 s), mean = 1.07 (CV = 16%); 4PL (300 s), mean = 1.12 (CV = 7.2%); spline (60 s), mean = 1.04 (CV = 3.7%); spline (300 s), mean = 1.04 (CV = 1.4%). The resulting post-analytical between-counting CV for the patient samples was used for evaluation (Fig. 1).

In the final study, the incubation time was increased from 2 to 3 h to reduce the time dependency of manual pipetting. Overall, improvements were achieved by increasing incubation time from 2 to 3 h, use of a logit-spline calibration function, and a counting duration of 5 min. To evaluate the improvements and the analytical performance, 7 laboratory technologists performed a total of 50 runs with 5 different lot numbers during a 12-month period.

Calibration of the TRAb assay was based on 6 concentrations: 0, 1, 2, 4, 16, and 40 IU/L. A high-concentration sample pool (C-high) was obtained from patients with a TSH concentration <0.01 mIU/L and a total thyroxine concentration >200 nmol/L. Lower-concentration sample pools were produced by diluting C-high with blood from male and female donors. Another pool made with blood from 200 male donors (T-pool) was measured in duplicate in the first 20 calibrations.

Analytical imprecisions (CVwithin, CVbetween, and CVtotal) for each pool were calculated to estimate functional assay sensitivity and linearity. Trueness was evaluated by use of WHO 90/672 at 3 concentrations: 1, 2, and 4 IU/L, respectively.

Reference intervals for our laboratory were estimated by measuring TRAb in duplicate in 126 healthy persons (34 pregnant women and 92 blood donors of both sexes).

Precision profiles for the 4 variants of the assay are illustrated in Fig. 1, which shows the reduction in noise (CV) by the expected factor √5 when changing from 1 to 5 min of counting time. The 2 calibration-fitting models (4PL and linear-logit-spline) gave fully equivalent results.

Fig. 1. Precision profiles for 4 variants of the TRAb assay.
Effect of curve-fitting model and counting time. Shown are examples of 2 different algorithms, 4PL and lin-log spline, at the recommended time (1 min) and as used in our laboratory (5 min). n = 10 countings.
in the range from 1.0 to 15 IU/L, but the linear-logit-spline function was the preferred model because of the better precision at concentrations <1.0 IU/L.

Precision data from 8 different serum pools are shown in Table 1. The functional sensitivity, estimated by increasing the dilution stepwise from 1:6 to 1:16, was ~0.75 IU/L.

The unexpected lack of linearity may reflect a low but undetectable concentration of antibody in the diluted samples; assuming a concentration of 0.1 IU/L eliminates the nonlinearity (Table 1). In 6 runs we measured WHO 90/672 in 3 known dilutions: 1, 2, and 4 IU/L. The mean values and [t × SE (P ~0.05, 2-tailed; df = 5)] were 0.84 (0.24), 1.97 (0.37), and 3.91 (0.35), respectively. There was no detectable bias at any concentration.

The upper reference limit (97.5 percentile) in the healthy individuals was 0.75 IU/L, and only 16% of the reference individuals had TRAb values above the manufacturer’s detection limit of 0.3 IU/L.

In our hands the functional assay sensitivity of the unmodified method was ~1.5 IU/L compared with 0.75 IU/L in the modified assay. Originally, the company designed the assay calibration function for a cutoff of 2.0 IU/L, covered by the calibration values 1.0 and 4.0 IU/L; however, the company later changed the cutoff from 2.0 to 1.5 IU/L, with a gray zone from 1.5 to 1.0 IU/L. Thus, 1.0 IU/L no longer functions as a low calibration value to stabilize the most important part of the calibration curve. Inclusion of a new lower point in the calibration function (e.g., 0.75 IU/L) would be helpful.

In conclusion, the functional sensitivity of the assay for TRAb could be changed from 1.5 to 0.75 IU/L because of longer incubation and counting times combined with a logit-spline model for calibration.

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References

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