References


Comparison of Thyrotropin-Receptor Antibodies Measured by Four Commercially Available Methods with a Bioassay That Uses Fisher Rat Thyroid Cells, Carol M. Preissner,1* Philip J. Wollhuter,2 John W. Sistrunk,2 Henry A. Homburger,1 and John C. Morris III 2,3 (1 Department of Laboratory Medicine and Pathology and 2 Division of Endocrinology, Department of Internal Medicine, Mayo Clinic, Rochester, MN 55905; * address correspondence to this author at: Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 1st St. SW, Rochester, MN 55905, fax 507-284-9785, e-mail preissner.carol@mayo.edu)

Quantification of thyrotropin-receptor antibodies is important in the diagnosis and management of patients with Graves disease (1). Antibodies with stimulating activity (TSI) have traditionally been detected in bioassays that measure their effect on cloned rat thyroid cells (FRTL-5) or on Chinese hamster ovary (CHO) cells transfected with recombinant human thyrotropin-stimulating hormone (TSH) receptor (2, 3). These assays can detect antibodies in up to 95% of untreated hyperthyroid Graves patients, but, with few exceptions (4), they require cell culture facilities and are labor intensive and time consuming.

As an alternative to bioassays, several methods have developed competitive immunoassays that measure the inhibition of the binding of labeled TSH by antibodies in patients’ sera. These methods use porcine TSH receptors and luminescence-labeled bovine TSH. The manufacturer’s literature cites a clinical sensitivity of almost 99% for the LUMItest® TRAK (TRAK) human assay (BRAHMS AG) uses human recombinant TSH receptors and luminescence-labeled bovine TSH. The manufacturer’s literature cites a clinical trial that achieved a diagnostic sensitivity of almost 99% with the research version of the DYNOtest® TRAK human assay (5).

We have been performing the TSI bioassay with FRTL-5 cells routinely for more than 15 years. The TSI test volumes have increased steadily over that time, requiring an ever-increasing number of assays each week. In 1998,
we added the Kronus® TRAb radioreceptor assay (TRAb) to our test menu to reduce the number of requests for TSI. During the preimplementation evaluation of the Kronus reagents, we found equivalent results in 80 of 89 random patient samples. Of the remaining nine samples, five were positive by TSI and not by TRAb, and four were positive by TRAb and not by TSI.

The availability of the BRAHMS reagents as well as two additional assays from Kronus led us to compare their diagnostic capabilities to those of our current TSI and TRAb assays. Under a protocol approved by the Institutional Review Board, two of us (JCM and JWS) collected 83 samples from patients seen in the Mayo Clinic Thyroid Clinic and stored the samples frozen. They were then analyzed with three TSH receptor antibody assays from Kronus [TRAb, Coated Tube RIA (CT), and ELISA], the BRAHMS TRAK assay, and the TSI assay. The laboratory personnel who performed the analyses were blinded to the diagnoses and to the results of the other assays until all of the data were compiled. The samples included 41 sera from patients with Graves disease (32 untreated hyperthyroid patients and 9 treated patients) and 42 sera from patients with other thyroid diagnoses, as follows: 13 with Hashimoto thyroiditis, 3 with nodules, 13 with multinodular goiter, 3 with cancer, 2 with silent thyroiditis, and 8 with no thyroid disease.

The assay sizes were determined by the TSI assay, which could accommodate 38 patient samples per assay. Sera thawed for the TSI assay were stored at 4 °C and analyzed in the four competitive immunoassays within 24 h. All tests were performed and results were calculated according to the manufacturers’ recommendations. For methods that provided calibrators (TRAb, CT, ELISA, TRAK), full calibration curves were used to calculate quantitative results. An alternative calculation, the qualitative index of inhibition of TSH binding, was also determined. The routine TSI assay was performed with cells passaged seven times. The FRTL-5 cells were first grown in supplemented Coon’s modification of F-12 medium for 10 days in 96-well microtiter plates with periodic medium changes. Immunoglobulin fractions from 500 µL of patient sera, normal control sera, and Graves control sera were prepared and were then allowed to react with the FRTL-5 cells for 2 h at 37 °C [modified (2)]. An in-house RIA was used to measure cAMP from well supernatants. A TSI value for each patient sample was calculated as an index relative to the mean value of cAMP produced by five normal pools analyzed in each run. The formula used was:

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\text{TSI index} = \frac{\text{cAMP patient (pmol)}}{\text{cAMP normal pool (pmol)}}
\]

All of the commercially available methods provided results in ≤4 h with serum volumes of 50–100 µL. The TRAb method was calibrated with MRC LATS B, whereas the others used WHO 90/672. TSH receptors were used either in liquid phase (TRAb) or adsorbed to tubes (CT and TRAK) or microtiter plates (ELISA). The TRAb and CT assays used radioactive labels; the ELISA used biotin-streptavidin peroxidase, and TRAK used a chemiluminescent signal.

The TSI assay detected antibodies in 94% of untreated Graves patients and 2% of the patients with other thyroid diagnoses (Table 1). This is similar to previously published results (2). In general, the TRAK assay gave results that were identical to the TSI, although one or two of the three calibrators in the critical area of the curve (0 IU/L, 1 IU/L, and 2 IU/L) had to be adjusted in all three of the TRAK assays. In each case, poor CVs (>15%) of the duplicate measurements required that one replicate be chosen to process the data. The eliminated replicates were easily identifiable as those points with relative light units (RLUs) that did not give a useable calibration curve because the mean RLUs did not consistently decrease with increasing concentrations of TSH receptor autoantibodies. It was not possible to calculate the data without making the adjustments. According to the manufacturer’s instructions, values <1 IU/L were to be regarded as negative and values ≥2 IU/L were to be regarded as positive; therefore, subjective manipulation of the low end of the curve could easily alter the interpretation of patient results. It is worth mentioning that the three assays from Kronus that were also calculated quantitatively did not require such changes to any of the calibrators when processing the data.

Relatively poor sensitivity (~70%) was seen for some of the methods. When we recalculated the data and considered those samples in the indeterminate range as positive, some of the methods showed a substantial improvement in sensitivity without any loss of specificity. This was seen with the TRAb assay that we are currently using. With a cutoff of 15% for the index, the TRAb assay detected <70% of the untreated Graves samples. When those samples with values of 10–14% were also interpreted as positive, the sensitivity increased to 88% while retaining 100% specificity. Other methods that showed similar improvement in sensitivity were the quantitative CT and ELISA assays. However, over time and with a larger population of patients, this occurrence may not hold true.

Overall, the TRAK reagents produced results most comparable to the TSI assay. In our hands, however, we thought it the hardest to maintain in our routine laboratory. We perform several in-house chemiluminometric assays that use triggering reagents from Bayer Corporation in the luminometers. The assay from BRAHMS uses its own triggers that would require flushing the reagent tubing before and after counting each TRAK assay. Likewise, the TRAK assay uses a wash buffer with a formulation that is different from the one we routinely use with the automated tube washers.

Of note, one serum from an untreated Graves patient
was negative in all of the assays. The clinical exam along with other laboratory and radiology tests supported the diagnosis. The other TSI-negative (just below the cutoff) Graves patient was positive in the TRAK assay and the ELISA index. In another patient, the TSI and TRAK assays were the only ones to detect antibodies. Most of the inconsistent results in the non-Graves samples seemed to occur randomly, except in two patients. One Hashimoto patient was positive in the TSI (at the cutoff concentration) and ELISA index, indeterminate in the ELISA quantitative and TRAK assays, and negative in the others. Similarly, a serum from a patient with thyroiditis was positive in the ELISA index and TRAK, indeterminate in the CT index and ELISA quantitative, and negative in the other assays.

We currently are performing the TSI and the TRAb index assays in our laboratory. There are still three to four times as many requests for TSI as for TRAb, despite the differences in cost and turnaround time. It is likely that some of these requests occur in cases in which it is important to distinguish whether the activity of the TSH-receptor antibodies is stimulatory or not. The TRAb and other commercially available assays offer slight differences in assay configuration and performance; each laboratory needs to evaluate the one that fits best into its workflow. Important issues such as calibrator reproducibility and determination of cutoff concentrations need to be carefully addressed to provide results comparable to those obtained from the established TSI assay.

The FRTL-5 cell-based bioassay that we use may be modestly less sensitive than that performed with recombinant TSH receptor expressed in CHO cells. We have not compared the two in the current evaluation, because our aim was to examine the binding assays directly. We have not, however, seen differences in performance of the FRTL-5 cell-based assay and CHO cells in informal evaluation (not published). Clearly, our current data suggest that modern binding-based assays rival bioassays in sensitivity in the sera from patients with Graves disease. Discrepancies may be seen more often in patients with primary hypothyroidism who may be more likely to have inhibitory antibodies that are not seen in the stimulatory bioassay. Our study and the samples included in it, however, were not adequate to evaluate this question.

The manufacturers generously provided all of the kits.

TABLE 1. Summary of patient results.

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<tr>
<th>Patients</th>
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a Results calculated using positive cutoff levels determined by manufacturers.
b Results considered positive if values were in the indeterminate or positive ranges.
c Number of positive results (% positive).
d ND, not determined.

References