Chemiluminescence Immunoassay of Thyrotropin with Acridinium-Ester-Labeled Antibody Evaluated and Compared with Two Other Immunoassays

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A new chemiluminoimmunoassay of thyrotropin (TSH) involves antibody labeled with acridinium ester ("Magic Lite System," Ciba Corning Diagnostic Corp.). The assay is rapid, with two incubations totaling 2.5 h, requires two standards per run, and takes 10 s per sample for the quantification step. Analytical performance, within- and between-run reproducibilities, and linearity were excellent. The detection limit is 0.04 milli-int. unit/L. Results correlated well with those obtained by immunoradiometric assay (RIA-gnost hTSH, Hoechst-Behring) and immunofluorometric assay (hTSH Delfia, LKB): r = 0.975. TSH measurements in 32 euthyroid subjects ranged from 0.4 to 4.8 milli-int. units/L (mean 1.35 milli-int. units/L). TSH values for 51 hypothyroid and subclinically hypothyroid patients ranged from 2 to 65 milli-int. units/L. TSH values for 33 hyperthyroid patients (<0.14 milli-int. unit/L, <0.04 milli-int. unit/L in 16 of the 33) were clearly lower than for most untreated euthyroid subjects. For 169 other individuals whose thyroid function was being routinely assessed, TSH ranged from 0.4 to 4.8 milli-int. units/L, three had TSH <0.14 milli-int. unit/L, and four had TSH between 0.14 and 0.4 milli-int. unit/L. This system is as efficient and reliable for screening for thyroid function as the two comparator systems.

Additional Keyphrases: immunochemiluminoimmunodiagnostic technique, immunoradiometric, and immunofluorometric assays compared—monoclonal antibodies—thyroid status—clinical sensitivity and specificity—two-point calibration—screening

In recent years, highly sensitive "sandwich"-type immunoassays, with use of monoclonal antibodies, have been developed for determining thyrotropin (TSH) concentrations.1 Such assays lowered the limit of detection and modified the strategy for routine thyroid examination. Particularly, the suppressed TSH concentrations associated with hyperthyroidism are more clearly distinguished from the lower limit of the euthyroid TSH range. These two-site procedures have been based on radiometric (1-4), fluorometric (5), immunoenzymometric (6), and chemiluminometric (7) assays.

Recently a commercially available immunochemiluminoimmunoassay kit has been introduced ("Magic Lite System"; Ciba Corning Diagnostics Corp., Medfield, MA) that combines the advantages of an acridinium-ester-labeled monoclonal antibody and a simplified separation procedure based on the use of magnetizable particles (8, 9). Here, we evaluate this system for use in measuring TSH in routine clinical practice and compare results with those by an immunoradiometric and an immunofluorometric assay.

Materials and Methods

Analytical Procedures

Immunochemiluminoimmunometric assay for TSH. The Magic Lite TSH assay includes two anti-TSH monoclonal antibodies, one immobilized on paramagnetic particles and the other directly labeled with acridinium ester (the signal reagent). Each is specific to different epitopes on the intact TSH molecule. In the assay, 100 μL of serum is incubated with 100 μL of "Lite" reagent (acridinium-ester-labeled antibody) for 2.5 h at room temperature. After addition of 500 μL of solid phase and incubation at room temperature for 30 min, bound and free labeled antibody are magnetically separated and the TSH is quantified at the rate of 10 s per sample. Oxidation of acridinium ester by alkaline hydrogen peroxide, automatically performed in the Magic Lite analyzer, initiates chemiluminescence emission. The manufacturer has informed us that, at the concentration used in the test (<5 parts per billion), the acridinium conjugate and the products of the chemiluminescence reaction have shown no mutagenicity in an Ames test as performed by an independent testing laboratory.

The light emission is expressed as photon counts accumulated during 2 s (relative light unit, RLU). Only two TSH calibrators (standardized against the second International Reference Preparation, 2nd IRP, coded 80/558) are required in each assay: one low calibrator (0 milli-int. unit/L) and one high calibrator (approximately 16 milli-int. units/L). Their RLU values in each assay are used to re-calibrate a manufacturer-generated Master Curve, which is stored in the analyzer's memory for each lot of reagents. The re-calibration takes place via algorithms in the analyzer software, which transform the spline of the Master Curve. We performed all assays of calibrators and unknown samples in duplicate.

Immunoradiometric and immunofluorometric assays for TSH. We compared results obtained with the Magic Lite TSH assay with those of an immunoradiometric assay (IRMA; "RIA Gn ost hTSH" from Hoechst-Behring, Frankfurt, F.R.G.) and with our routine test, the immunofluorometric assay (IFMA; "hTSH Delfia" from LKB, Bromma, Sweden). These two methods are also "sandwich"-type techniques involving two monoclonal antibodies. Standards are calibrated against the 2nd International Reference Preparation for the IRMA and against the First International Reference Preparation, 1st IRP coded 68/38, for the IFMA.

Other assays. Concentrations of free thyroxin (FT4) and free triiodothyronine (FT3) were measured with a chromatographic method (Sclavo, Siena, Italy). Normal reference intervals for FT4 and FT3 were 8 to 15 pmol/L and 4 to 7 pmol/L, respectively.

Clinical Evaluation

Patients. The population we studied comprised all subjects who were being routinely assessed for thyroid function during two months (n = 388). In each case the diagnosis was established on the basis of clinical examination, measure-
ments of FT3, FT4, basal TSH (by IFMA, and, if necessary, the TSH response 30 min after intravenous administration of thyrotherbin (TRH). Most (304) of these patients were euthyroid. The 128 of these who were being treated (with levothyroxine, neomercazole, etc.) on the day of examination were excluded from the clinical validation. The 176 others (group 1) were new patients or subjects with a known history of thyroid disease but not receiving treatment: 54 had a simple goiter, 45 a multinodular goiter, 34 a solitary thyroid nodule, one a cyst, two a pituitary deficiency, and eight had Graves' disease; 32 who had no evidence of thyroid disease constituted the reference population (group 0).

In 33 patients (group 2) there was evidence of hyperthyroidism: increased concentrations of FT3 and FT4 and an absent response of TSH to TRH. Graves' disease was diagnosed in eight patients (two treated with neomercazole); toxic multinodular goiter in 10 (one being treated with neomercazole); solitary toxic nodule in five; Hashimoto's thyroiditis in three; and two cases of hyperthyroidism were induced by amiodarone and five by an excess of levothyroxine. Of the 33 hyperthyroid patients, four were considered to have subclinical hyperthyroidism: they had normal or borderline values for FT3 and (or) FT4 and a very low (<1 milli-int. unit/L) TSH response to TRH.

Hypothyroidism (above-normal TSH and low FT3 and FT4 concentrations) or subclinical hypothyroidism (normal values for FT3 and FT4, and high TSH response to TRH, >20 milli-int. units/L) was diagnosed in 51 patients (group 3); in this group, 11 patients were being treated with levothyroxine and 20 were post-surgical hypothyroid patients.

Results

Analytical Variables

Precision. For evaluation of precision within- and between-run, we prepared pools of human serum with TSH concentrations ranging from 0.05 to 29 milli-int. units/L. As expected, the CVs are highest for the lowest concentrations of TSH. Intra-batch CVs (n = 10) were 12%, 7%, 5%, 4.5%, 2.5%, 2.5%, and 3% for TSH values of 0.06, 0.34, 1.22, 6.8, 13.4, and 29 milli-int. units/L, respectively. Inter-batch CVs (n = 23) were 57%, 15%, 9.5%, and 9% for TSH values of 0.04, 0.18, 2.8, and 13.7 milli-int. units/L, respectively. We observed similar results with IRMA and IFMA (Figure 1).

Detection limits. By extrapolating the intra-assay precision profile \( \Delta C = f(C) \) (absolute error vs concentration, with \( \Delta C = 2 \ SD \)), we derived the limit of detection as 0.01 milli-int. unit/L. We observed the same value when a low-TSH sample (0.3 milli-int. unit/L) was diluted with "zero"-TSH serum from a hyperthyroid patient (Figure 2). When estimated from the inter-assay precision profile, the limit of detection was 0.04 milli-int. unit/L. This latter value is more consistent with the conditions obtaining in a routine laboratory (different lots of reagents, different technicians, etc.). Detection limits obtained under the same conditions by the IRMA and IFMA methods were also about 0.01 milli-int. unit/L (intra-assay) and 0.04 milli-int. unit/L (inter-assay).

Linearity. Three patients' samples, containing 104, 0.11, and 0.3 milli-int. units/L, when serially diluted with serum from a hyperthyroid individual, gave log-linear responses to dilution down to the limit of detection.

Analytical recovery. This was assessed by assaying a sample (TSH concentration, 0.87 milli-int. unit/L) with concentrations of added thyrotropin ranging from 0.1 to 20 milli-int. units/L (standard WHO 80/558, purchased from the National Institute for Biological Standards and Control, London, U.K.). The mean percentage recovery of added thyrotropin was 101% (extreme values: 96%, 105%).

Intra-assay drift. No significant intra-assay drift (Student's paired t-test: \( t = 0.12, n = 10, \) not significant) was observed when the same sample was positioned at the beginning and the end of 10 consecutive runs containing up to 100 tubes each.

Matrix effect. Moderately hemolyzed samples (2.8 to 13.6 g of hemoglobin per liter) averaged slightly higher TSH values for percentage recovery than unhemolyzed: (hemolyzed TSH/unhemolyzed TSH) \( \times 100\% = 112\% \) (SD 13%; \( n = 16 \)). There was no correlation between increasing hemoglobin concentration and increased TSH (recovery, \( % = 0.38 \ Hb + 110, r = 0.08, n = 16 \)). No change in TSH concentration was observed between hemolyzed and unhemolyzed "zero"-TSH serum. However, hemolysis reflects mistreatment of the sample and results should be interpreted with caution. Triglyceride (final concentration, 30 g/L) or bilirubin (300 mg/L) introduced into "zero"-TSH serum from three hyperthyroid subjects had no significant effect on the TSH values. With samples containing TSH in concentrations from 0.47 to 99.5 milli-int. units/L, there was a slight under-recovery of TSH in both the triglyceride-supplemented
Fig. 2. Count rate vs expected TSH values for a sample diluted with the “zero”-TSH serum of an hyperthyroid patient. The limit of detection is about 0.01 milli-int. units/L, the point of intersection between the dilution curve and the hyperthyroid serum count-rate line.

(mean % recovery = 92%) and bilirubin-supplemented (mean % recovery = 97%) samples.

“Two-point calibration” validity (Figure 3). We measured TSH concentrations in 63 samples in two different runs. The first was performed using the Magic Lite “two-point calibration” system. In the second assay, the standard curve was obtained from an included-in-the-run set of seven TSH standard preparations (range 0 to 100 milli-int. units/L) supplied by Ciba Corning. Identical results were observed by the two methods for TSH concentrations below 5 milli-int. units/L; the regression equation for the relation was $y = 0.93x - 0.01$ (Student’s paired t-test, t = 1.5; not significant). For TSH >5 milli-int. units/L, the results by the “two-point calibration” system were significantly higher than those obtained with the full set of standards ($y = 1.24x - 1.44$, t = 3.3; P <0.01).

Comparison with IFMA and IRMA (Figure 4). Basal TSH concentrations for the 388 subjects were assayed by the three methods. Linear regression analysis for TSH concentrations as measured with the ICMA ($y$), IRMA ($x$), and IFMA ($x'$) methods gave $y = 1.04x - 0.11$ ($r = 0.975$, n = 371, $P <0.001$) and $y = 1.26x' + 0.09$ (n = 388, r = 0.975, $P <0.001$). The correlation coefficients confirmed the evidently good agreement between the methods. But: (a) ICMA and IRMA methods gave identical values, whereas ICMA results were higher than IFMA values (slope of regression line: 1.26); (b) there were no widely discrepant results between ICMA and IRMA. In contrast, two sera measured 7.2 and 8.4 milli-int. units/L by IFMA, 1 and 0.33 milli-int. units/L by ICMA, and 1.5 and 0.9 milli-int. units/L by IRMA. The two IFMA results were falsely increased by the presence of anti-IgG antibodies in the patients’ sera, as demonstrated by addition of nonimmune mouse serum, after which these sera gave on re-assay 1.2 and 0.4 milli-int. units/L, respectively.

Clinical Evaluation

The distributions of basal TSH concentrations in the reference population (group 0) were log-normal by each method. Reference TSH values established from the 95% confidence interval were 0.4 to 4.8 milli-int. units/L (mean 1.35 milli-int. units/L) by ICMA, 0.3 to 3.5 milli-int. units/L (mean 1 milli-int. units/L) by IFMA, and 0.45 to 4.6 milli-int. units/L (mean 1.4 milli-int. units/L) by IRMA.

The distribution of TSH ICMA concentrations in euthyroid (group 1), hyperthyroid (group 2), and hypothyroid populations (group 3) is shown in Figure 5.

Of the 33 hyperthyroid patients, 16 had basal TSH...
concentrations below the limit of detection (0.04 milli-int. unit/L), while the others ranged from 0.04 to 0.14 milli-int. unit/L; values for the three patients treated with neomycin were 0.05, 0.09, and 0.14 milli-int. units/L.

Basal TSH concentrations of the 51 hypothyroid patients ranged between 2 and 65 milli-int. units/L; 13/51 were within the euthyroid reference interval, their hypothyroid state was confirmed by an exaggerated response to TRH stimulation (>30 milli-int. units/L); 10 of them were subclinically hypothyroid. Seven low TSH concentrations (below the lower limit of the euthyroid reference interval) were observed in the euthyroid population (group 1).

The same kind of distributions were observed with IFMA and IRMA (Figure 6). However, slight differences with ICMA can be pointed out: (a) In 17 of 33 hyperthyroid patients, data were above the detection limit by ICMA, with 2/33 and 4/29 above the detection limits by IFMA and IRMA, respectively. Technical errors were excluded by repeating samples in another assay. The TSH stimulation test was performed for eight of these 17 patients: five gave a low but significant response (i.e., TSH >0.04 milli-int. unit/L); only 2/8 gave significant responses with the two other methods. (b) Of the 14/176 (IFMA) and 7/174 (IRMA) euthyroid subjects with low basal TSH concentrations, three (IFMA) and two (IRMA) were below the limit of detection. In contrast, of the seven low TSH euthyroid subjects by ICMA, none showed an undetectable concentration of TSH. Such differences could suggest that the ICMA assay measures "TSH fractions" undetectable by the two other techniques. Further investigations will be required to confirm these results.

Discussion

We found the immunochemiluminescent assay of TSH easy to use and reliable. In the Magic Lite assay, the chemiluminescent compound is directly attached to an antibody; there is no need for a secondary reaction, such as an enzymatic one, to initiate the chemiluminescent signal. The long counting time of a sensitive immunoradiometric assay is not required because of the high specific activity of the chemiluminescent-labeled analyte. Each labeled molecule potentially generates one "event" (one photon emitted). In practice, only about 10% of these emit light. However, this is much greater than with 125I labeling, where there is one event per second for 7.5 x 10⁶ molecules of label. With the Magic Lite system, full standard curves are eliminated; only a simple two-point calibration is necessary. We found, in a single experiment, that results obtained by using two-point calibration compared well with results obtained by using full standardization for TSH concentrations <5 milli-int. units/L. We found also that this system gives a slight overestimation for TSH concentrations >5 milli-int. units/L; this is obviously without clinical repercussion because basal TSH concentrations >5 milli-int. units/L correspond to hypothyroid patients. The two-point calibration system simplifies every assay and cuts down on reagent use and operator hands-on time. Small runs can be made cost effective to generate results for just a few patients.

Our analytical evaluation indicated excellent performances of the immunochemiluminescent system for TSH: within- and between-run reproducibilities, limit of detection, linearity, recovery, no matrix effect, and good correlations with the IRMA and IFMA methods. The lower results we obtained by the IFMA assay may be attributed to differences in the calibration system for the TSH standards.

Clinical evaluation showed that TSH by ICMA is a valuable tool in the assessment of hyperthyroidism. Taking 0.2 milli-int. unit/L as the upper TSH concentration in hyper-
thyroidism, the clinical sensitivity of the TSH ICMA was 100% (TSH <0.2 milli-int. unit/L for all of our hyperthyroid patients) and its clinical specificity was 98% (percentage of euthyroid subjects with a basal TSH >0.2 milli-int. unit/L).

We conclude that the immunochemiluminometric assay from Ciba Corning Diagnostics is accurate and precise for the measurement of TSH. This "Magic Lite System" is efficient and reliable for use in routine screening for thyroid function.

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Improved Measurement of Urinary Total Protein (Including Light-Chain Proteins) with a Coomassie Brilliant Blue G-250–Sodium Dodecyl Sulfate Reagent

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The Coomassie Brilliant Blue G-250 method for urinary proteins underestimates urinary immunoglobulin light chains when albumin or pooled serum is used as the protein standard. The specific color yields of these and other pro-
teins can be brought closer together by adding sodium dodecyl sulfate to the reagent; however, there is some loss of sensitivity. We found such a reagent to be satisfactory for assayig urinary proteins on studying 43 patients with light-chain proteinuria, 19 of whom had multiple myeloma and six possible multiple myeloma.

Additional Keyphrases: multiple myeloma • proteinuria • myeloma kidney • optimization by the simplex technique

Of the many procedures described for assay of urinary total proteins, the Coomassie Brilliant Blue G-250 (CBB) method has significant advantages. It is extremely simple to per-
form, requiring only the addition of sample to the reagent. In contrast to the method of Lowry et al. (1), the CBB procedure for urinary proteins is affected by few interferents. Of a long list of compounds tested (2), only toltbutamide, at high concentrations, and hemoglobin falsely increased the protein; hydrochloric acid invalidated the test, yielding greatly reduced results. Bradford (3) also tested several potential interferents; he found results were affected by the presence of detergents and some other compounds, none of them endogenous.

The analytical sensitivity of the CBB method can be maximized by increasing the concentration of CBB to just below its limit of solubility (4). The sensitivity is restricted by the low solubility of the dye, which is about 180 mg/L in a solution that contains 1.6 mol of H2PO4 and 0.8 mol of ethanol per liter (4). Most sources of CBB contain fillers; a solution containing 125 mg of CBB, 1.5 mol of H2PO4 and 0.8 mol of ethanol per liter should have an absorbance of about 0.5 A at 450 nm. The concentration of CBB can be adjusted to correspond to this absorbance (5). Increasing the concentration of alcohol increases the solubility of the dye, but it also causes an undesirable and progressive shift to the blue form of the dye. This alcohol-induced shift can be reversed by adding more H3PO4, but this reduces the specific color yield (4). With a more concentrated reagent, urinary protein concentrations as low as 2 mg/L can be determined (4).

Disadvantages of the method includes a slow precipitation of CBB with consequent loss of analytical sensitivity during storage. The most serious problem with the CBB method for clinical use is the lower specific color yields for immunoglobulin light chains as compared with albumin. Thus the Bradford method (3), and variants thereof (2), underestimate light-chain proteinuria—which is the only laboratory evidence of multiple myeloma in some patients (6, 7). Light chains may cause acute tubular necrosis and so-called "myeloma kidney" (8). Although no correlation was found between the amount of light-chain protein excreted and prognosis of patients with myeloma, early detection and treatment may delay the onset of myeloma kidney (9).

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