Henry Ford Hospital Medical Journal

Volume 35 | Number 4 Article 9

12-1987

# Comparison of Four High-Sensitivity Immunoradiometric Assays for Thyrotropin and Results of Preliminary Clinical Studies

Malachi J. McKenna

Earl Goad

Mohini Pimputkar

Carolyn S. Feldkamp

# Comparison of Four High-Sensitivity Immunoradiometric Assays for Thyrotropin and Results of Preliminary Clinical Studies

**Malachi J. McKenna, MD,* Earl Goad, BS,† Mohini Pimputkar, MS,† and Carolyn S. Feldkamp, PhD†**

*New immunoradiometric assays (IRMAs) that detect low concentrations of thyroid-stimulating hormone (TSH) have recently become available for routine diagnostic use. These new assays have the putative advantage over conventional radioimmunoassay in that they can distinguish hyperthyroidism from euthyroidism by the finding of a serum TSH below the normal limit. In the present study we sought to evaluate four of these kits according to analytical performance characteristics and clinical utility. All IRMAs could detect TSH at a concentration substantially below the lower limit of normal and thus effectively identify hyperthyroid samples. Although differences in the performance characteristics were found, all assays were clearly superior to the conventional radioimmunoassay. It is recommended that IRMAs for TSH be used as routine diagnostic tests for thyroid dysfunction. Their full value in the assessment of hyperthyroidism and other thyroid disorders has yet to be determined. (Henry Ford Hosp Med J 1987;35:201-6)*

Functional disorders of thyroid gland activity, both oversecretion and undersecretion, are readily detected by the use of radioligand assays. In current clinical practice a diagnosis of hyperthyroidism is most often confirmed by the finding of an elevated free thyroxine index (FTI), which is the product of serum total thyroxine and triodothyronine resin uptake. Hypothyroidism is identified by a low FTI, and an elevated thyroid-stimulating hormone (TSH) level distinguishes primary disease from hypothalamic-pituitary hypofunction. In circumstances of clinical doubt, the TSH response to thyrotropin-releasing hormone (TRH) can clarify the diagnosis: being suppressed in primary hyperthyroidism, hyperresponsive in primary hypothyroidism, and hyporesponsive in secondary hypothyroidism.

It has recently been suggested that the laboratory approach to diagnosis of thyroid dysfunction should change with the introduction of immunoradiometric assays (IRMAs) that are of high sensitivity (1,2). Their ability to detect TSH at much lower concentrations than standard radioimmunoassays (RIAs) means that TSH in serum can be detected well below the reference range for healthy persons, a limitation of all previously available methods. Therefore, a single specimen of blood for measurement of TSH by the IRMA technique should be an appropriate screening test for states of both reduced and augmented TSH secretion.

In the present study we sought to 1) compare the performance characteristics of four IRMAs for TSH regarding sensitivity, precision, and accuracy; 2) determine reference ranges; and 3) evaluate the capability of these assays to distinguish hyperthyroidism from euthyroidism.

## Methods

### Assays

Four new high-sensitivity IRMA kits for TSH were evaluated (Table 1): NML TSHIRMA (Organon Teknika, Durham, NC), Echoclonal TSH (Bio-Rad Laboratories, Hercules, CA), MAGIC mab TSH (Ciba Corning, Medfield, MA), and Allegro HSTSH (Nichols Institute Diagnostics, San Juan Capistrano, CA). According to the test principle for IRMA, an excess of radioactively labeled antibody reacts with the full amount of unknown antigen. Two or more antibodies (at least one of which is monoclonal) bind to different antigenic sites on the TSH molecule, resulting in an antibody-thyrotropin-antibody "sandwich" (Fig 1). In contradistinction, in the RIA the unknown antigen competes with radioactively labeled antigen for binding with a limited quantity of antibody. This methodological difference, in

Submitted for publication: July 8, 1987.
Accepted for publication: September 8, 1987.
*Formerly Department of Internal Medicine, Division of Endocrinology, Henry Ford Hospital. Currently Joslin Diabetes Center, Boston, MA.
†Department of Pathology, Division of Immunopathology, Henry Ford Hospital.
Address correspondence to Dr. McKenna, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215.

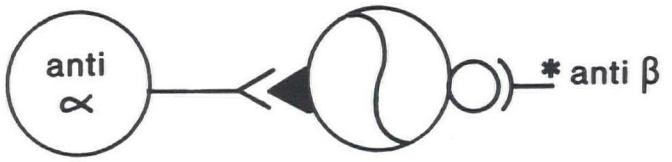


*Fig 1—Schema depicting principle of the IRMA technique whereby the TSH molecule is "sandwiched" between two antibodies, one binding to the α chain and the other (which is radiolabeled*) to the β chain.*

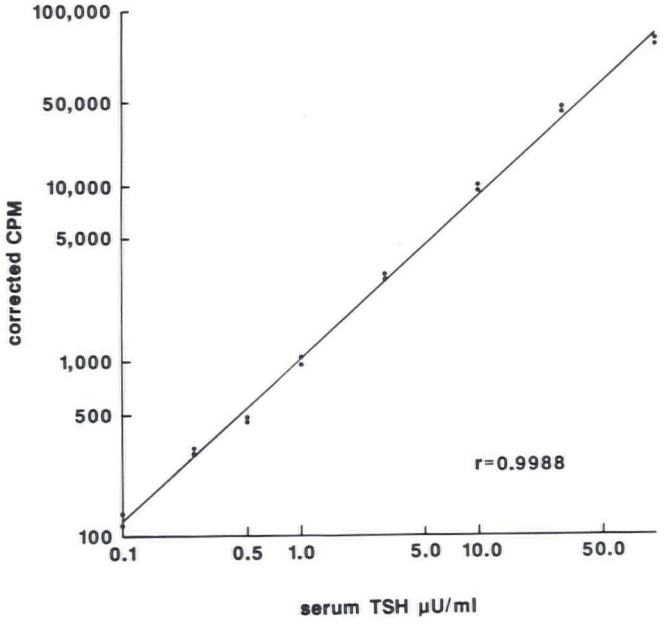


*Fig 2—Example of an IRMA standard curve from a Nichols assay. The very low standards (0.1 and 0.25 μU/mL) were obtained by dilution of the 0.5 standard with zero standard. The power fit gives a linear relationship between 0.1 and 100 μU/mL.*

addition to the use of monoclonal antibodies, permits greater sensitivity in the detection of TSH by IRMAs compared to conventional RIAs. Each of the four IRMAs were similar in that one or more monoclonal antibodies were employed and in two instances were radiolabeled (Table 1). Separation of antibody-bound antigen from free antigen was achieved by a variety of techniques: use of antibody-coated tubes (NML and Bio-Rad), binding of antibody coupled with biotin to avidin-coated beads (Nichols), and magnetic separation for which paramagnetic particles are covalently bound to one antibody (Corning). Assays were of similar duration with each requiring one or more wash steps. Samples were also measured by our standard RIA (Leeco, Southfield, MI).

For each assay, standards were analyzed in duplicate. In addition, the low standard was diluted with zero standard to give concentrations below 0.15 μU/mL. Adjustment for nonspecific binding was made by subtraction of the mean counts per minute

**Table 1**
**Immunoradiometric Assays**

| Assay | Number of Antibodies | | Separation Method |
|---|---|---|---|
| | Monoclonal | Polyclonal | |
| NML | 1 | 1* | Coated tube |
| Bio-Rad | 2* | 0 | Coated tube |
| Nichols | 2* | 1 | Avidin-coated bead |
| Corning | 1 | 1* | Magnetic |

*Radiolabeled.

**Table 2**
**Least Detectable Dose of TSH**

| | |
|---|---|
| NML | 0.09 μU/mL |
| Bio-Rad | 0.21 μU/mL |
| Nichols | 0.08 μU/mL |
| Corning | 0.04 μU/mL |

(CPM) of the zero standard from the average CPM of each standard. The standard curve of corrected CPM (the "y" axis) versus the known concentration of the TSH standards (the "x" axis) was calculated according to a power fit using a programmable calculator (HP41 CX) (Fig 2). The regression equation was then used to determine TSH concentrations in unknown analytes from their corrected CPMs.

### Performance characteristics

Sensitivity was evaluated by measuring the precision of multiple samples of the zero standard (n = 10) in an assay. The minimal detectable dose was defined as the concentration exceeding the zero standard at the 95% confidence level. Intraassay precision was evaluated using pooled sera at four different levels of concentrations, including a hyperthyroid pool for the very low end of the standard curve (FTI = 20.5 μg/dL). Interassay precision was evaluated for the Corning and Nichols assays. Accuracy at low levels of TSH concentration was analyzed by means of dilution and recovery studies. A precision profile was obtained for the Corning and Nichols assays by calculating the median of the coefficient of variation for duplicate determinations ranging from the limit of detectability to 10 μIU/mL. The Nichols and Corning assays were selected at an early stage of the evaluation for more detailed analysis in view of preliminary findings and convenience in the laboratory.

### Clinical studies

Nineteen clinical samples were analyzed in all four assays. Samples were randomly selected from laboratory specimens on the basis of either hyperthyroxinemia (an FTI above the 95% reference range, ie, > 11.5; n = 9) or euthyroxinemia (an FTI result within 1 SD of the mean for healthy adults; n = 10). A larger number of clinical samples (n = 28) obtained from subjects with hyperthyroidism, either due to Graves' disease (n = 11) or excessive thyroid hormone supplementation (n = 17), were analyzed in both the Corning and Nichols assays. The log-mean ± SD of the FTI was 16.6 ± 1.3 μg/dL, ranging from 11.1 to 31.8 μg/dL. In addition, samples from healthy, unmedicated adults (28 women, 27 men) were measured in the Corning, Nichols, and Leeco assays. A further 43 miscellaneous samples were measured in both the Corning and Nichols assays.

**Table 3**
**Intraassay Precision**

| Assay | Number per Assay | Mean Value (μU/mL) | Coefficient of Variation (%) |
|---|---|---|---|
| | | | (Low; High)* |
| NML | 2 | 1.2 | — (1.06; 1.40) |
| | 2 | 6.4 | — (6.45; 6.39) |
| | 2 | 16.3 | — (15.5; 17.1) |
| Bio-Rad | 3 | 1.7 | 3.8 |
| | 4 | 10.7 | 0.8 |
| | 4 | 29.6 | 3.3 |
| Nichols† | 4 | 1.7 | 3.8 |
| | 4 | 10.8 | 3.3 |
| | 4 | 30.4 | 2.6 |
| Corning† | 4 | 1.2 | 6.3 |
| | 4 | 9.1 | 3.5 |
| | 4 | 29.6 | 3.7 |

*The number of samples from the NML assay was insufficient to calculate a coefficient of variation.
†Average of four different assays.

**Table 4**
**Interassay Precision**

| Assay | Number of Assays | Mean Value (μU/mL) | Coefficient of Variation (%) |
|---|---|---|---|
| Nichols | 4 | 1.7 | 5.8 |
| | 4 | 10.8 | 4.6 |
| | 4 | 30.4 | 6.3 |
| Corning | 4 | 1.2 | 3.8 |
| | 4 | 9.1 | 1.7 |
| | 4 | 29.6 | 5.2 |

All data were log-transformed before statistical analysis. Linear regression analysis was performed by the least squares method, thereby giving a power fit for the log-transformed data.

## Results

### Performance characteristics

The minimal detectable dose of TSH was less than 0.1 μU/mL in all but the Bio-Rad assay which gave a result of 0.21 μU/mL (Table 2). The intraassay precision at low, medium, and high concentrations of TSH was similar in all assays; the number of samples for the NML assay was insufficient to calculate a coefficient of variation (Table 3). Reproducibility of measurements at very low TSH concentrations could not be determined because the value for the hyperthyroid pool was below the least detectable dose. Interassay variation was similar in both the Corning and Nichols assays (Table 4). Analysis of precision profiles for Corning and Nichols assays showed a similar pattern with a nadir at 1.0 μU/mL (Fig 3). Regarding the dilution studies measuring TSH at concentrations below 0.5 μU/mL, the percent recovered approximated 100% except for Corning which gave a result of about 65% (Table 5). Low recoveries were attributed to a matrix difference between the sample and the specially treated zero standard used as diluent. Recovery of added TSH at a low concentration of < 1.0 μU/mL yielded increased results with Bio-Rad, low values with NML and Corning, and satisfactory results with Nichols (Table 6). Recovery at higher concentrations gave good results for all assays.

**Table 5**
**Dilution Studies at Low Concentration of TSH**

| Assay | Dilution Factor | Expected Value A (μU/mL) | Measured Value B (μU/mL) | % Recovery B/A × 100 |
|---|---|---|---|---|
| NML | undiluted | 0.51 | — | — |
| | 1:2 | 0.26 | 0.32 | 123 |
| | 1:4 | 0.13 | 0.11 | 85 |
| Bio-Rad | undiluted | 1.48 | — | — |
| | 1:2 | 0.74 | 0.64 | 86 |
| | 1:4 | 0.37 | 0.39 | 105 |
| Nichols | undiluted | 1.03 | — | — |
| | 1:2 | 0.52 | 0.56 | 108 |
| | 1:4 | 0.26 | 0.31 | 119 |
| Corning | undiluted | 0.84 | — | — |
| | 1:2 | 0.42 | 0.28 | 67 |
| | 1:4 | 0.21 | 0.13 | 62 |

**Table 6**
**Recovery of Added TSH at Low Concentrations**

| Assay | Base B (μU/mL) | Added A (μU/mL) | Measured C (μU/mL) | % Recovery (C − B)/A × 100 |
|---|---|---|---|---|
| NML | 0.19 | 0.20 | 0.35 | 80 |
| | 0.19 | 0.74 | 0.88 | 93 |
| Bio-Rad | 0.21 | 0.20 | 0.27 | 30 |
| | 0.21 | 1.00 | 1.62 | 141 |
| Nichols | 0.02 | 0.20 | 0.24 | 110 |
| | 0.02 | 0.65 | 0.64 | 95 |
| Corning | 0.09 | 0.20 | 0.28 | 95 |
| | 0.09 | 0.80 | 0.62 | 66 |

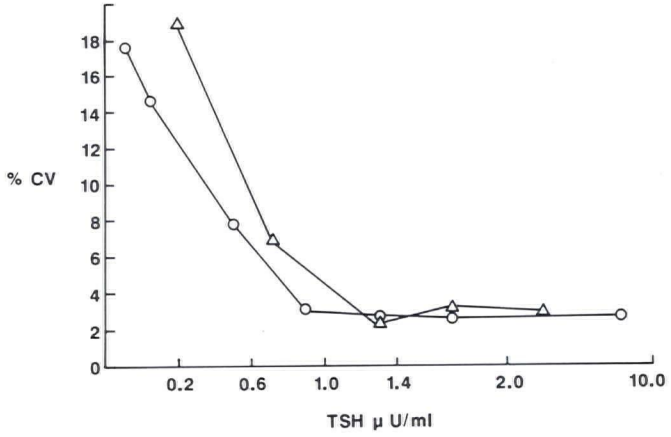


*Fig 3—Precision profile analysis for Nichols (△) and Corning (○) assays.*

### Clinical studies

The 19 clinical samples measured in the four assays gave similar results. Hyperthyroxinemic samples were lower than euthyroxinemic samples; this was not observed with the RIA (Fig 4). There was good agreement between all assays with correlation coefficients exceeding 0.95 (Table 7). The slope of the regression line for Corning and Nichols was closest to the line of

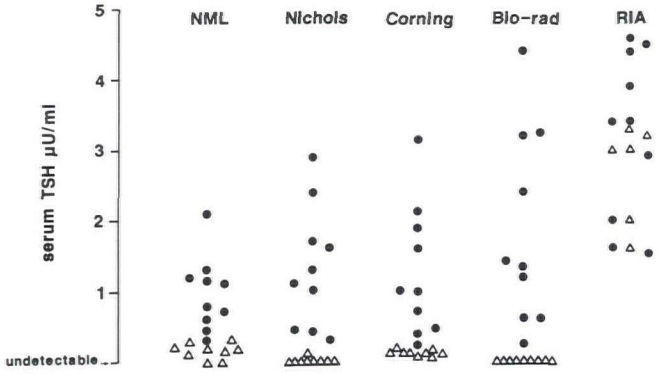


*Fig 4—Serum TSH values in nine hyperthyroxinemic (△) and ten euthyroxinemic (●) subjects.*

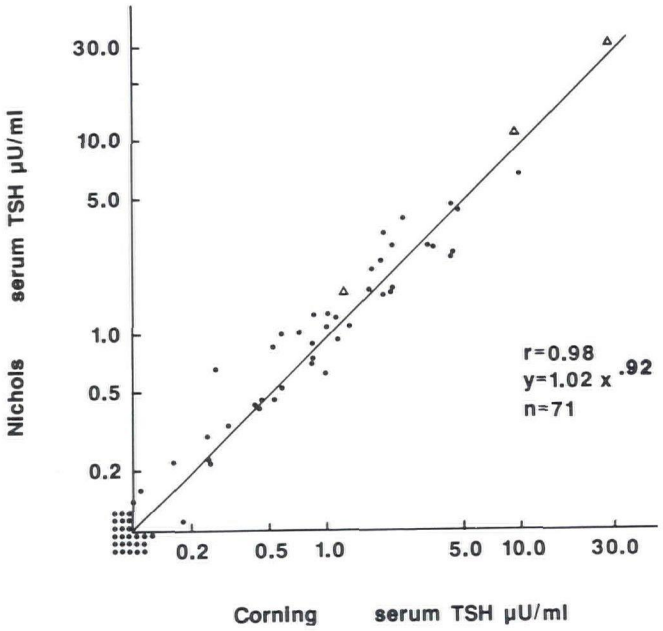


*Fig 5—Comparison of results obtained by Corning and Nichols assays. The average results of quality control specimens at three concentrations are represented by triangles.*

equivalence at 1.02. In an even larger sample (n = 71), this close relation was confirmed (Fig 5). The log-mean value (and 95% reference range) for a group of 55 healthy, nonmedicated adults with Corning was 0.74 µU/mL (0.19 to 2.79 µU/mL) and with Nichols was 1.78 µU/mL (0.59 to 5.21 µU/mL) (Fig 6). The findings were similar to the manufacturers' ranges of 0.29 to 5.11 µU/mL (Corning) and 0.9 to 4.6 µU/mL (Nichols). No sex difference was observed with both Corning and Nichols assays. However, values were significantly higher in women than men with the RIA, suggesting reduced specificity in women possibly due to higher serum gonadotrophins that could cross-react with the TSH antibody. All 28 samples from hyperthyroid subjects

**Table 7**
**Comparison of the Four Assays Using Clinical Samples (N = 19)**

| Assays x | | y | Correlation Coefficient (r) | Slope b | Intercept a |
|---|---|---|---|---|---|
| NML | vs | Bio-Rad | 0.88 | 1.47 | 1.46 |
| NML | vs | Nichols | 0.86 | 1.11 | 1.20 |
| NML | vs | Corning | 0.89 | 1.07 | 1.18 |
| Bio-Rad | vs | Corning | 0.98 | 0.77 | 0.80 |
| Bio-Rad | vs | Nichols | 0.98 | 0.78 | 0.84 |
| Corning | vs | Nichols | 0.97 | 1.02 | 1.11 |

Regression equation according to a power fit whereby $y = a \cdot x^b$. The line of equivalence has a slope of 1.0 and intercept of 1.0.

were below reference ranges for both Corning and Nichols assays. In contrast, only one of 23 of the same samples was below the euthyroid range for the RIA (Fig 7).

## Discussion

This study demonstrates that TSH levels can be detected at low concentration by all the IRMAs selected for comparison. Unlike a conventional RIA, the IRMAs could readily detect TSH below the lower limit of the normal range. In so doing, the new assays can distinguish hyperthyroidism from euthyroidism.

Differences in the performance characteristics between the four assays were observed. Sensitivity results, although variable, were comparable in that the limit of detectability in each case was nearly tenfold lower than the bottom of the expected normal range. Differences in accuracy, reflected in both the parallelism and recovery studies, were also documented at low TSH concentrations (Tables 5 and 6). Again, these findings are probably of little clinical relevance. Of greater importance is that all four assays distinguished hyperthyroxinemic samples from euthyroxinemic ones satisfactorily and to the same degree (Fig 4). In more detailed clinical studies with Corning and Nichols assays, it was possible in all instances to identify cases of hyperthyroidism by virtue of a single TSH measurement. Indeed, values were markedly suppressed below the euthyroid range (Fig 7). This is in accordance with other published reports (1,3-7).

Though not the focus of this report, IRMAs can also measure TSH accurately in the euthyroid and hypothyroid ranges. However, unlike RIAs, IRMAs are theoretically subject to error at very high levels as a consequence of the so-called "hook" phenomenon (8). The high-dose hook effect may occur when the analyte concentration approximates antibody concentration. An excessive quantity of antigen binds to a single antibody; these incomplete complexes do not separate with the antibody-antigen-antibody complexes. Thus, counting rates decrease inversely proportional to the quantity of analyte. This phenomenon occurs only at concentrations exceeding 100 µU/mL; thus, very high levels of TSH may be underestimated. This aberrancy is probably of quantitative rather than qualitative significance: a TSH value above 100 µU/mL is rare and usually associated with florid clinical signs of hypothyroidism. As yet, there is not a single report of this phenomenon eventuating in an

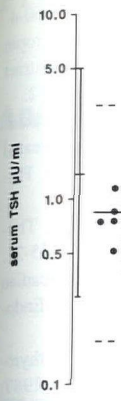


*Fig 6—Seru*
*(●) and men*
*lines. Expec*
*RIA gave sig*
*(p < 0.005)*

erroneous di
tified by mea
Another p
the method o
Methods for
oratory, are
computerizat
quality contr
measuring T
stances of us
have a superi
mal values at
The impac
tained, but th
chemical int
advantages h
detect situat
blood sample
perthyroidism
$T_3$, or the free
not, and shou
ciated with h
autonomous
hypothyroid
IRMAs will
to confirm du
situations wh
suppressive th
the IRMA ra
formation (11
elucidating th
(9,12,13). In
diurnal patter

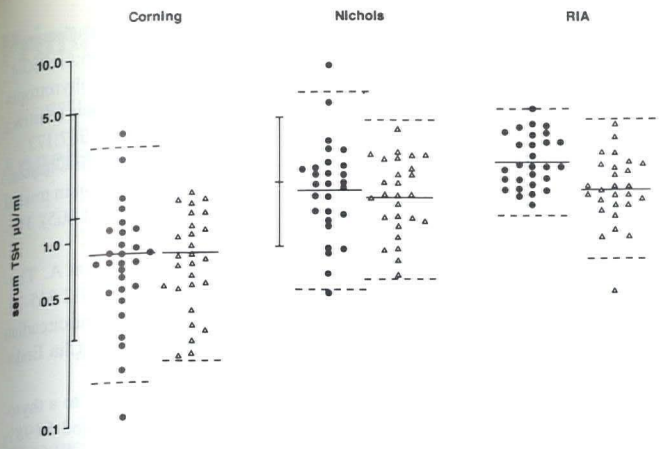


*Fig 6—Serum TSH values in healthy adults including women (●) and men (△). The mean ± 2 SD is represented by horizontal lines. Expected 95% ranges are indicated by vertical lines. The RIA gave significantly higher values in women compared to men ($p < 0.005$).*

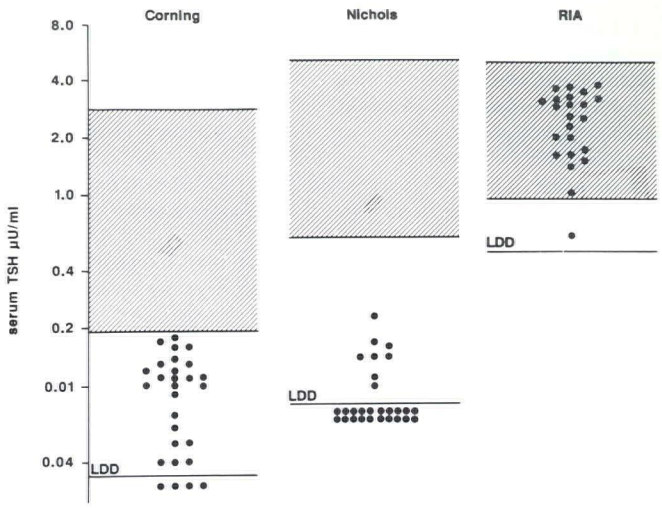


*Fig 7—Serum TSH values in hyperthyroid subjects plotted with regard to the 95% reference ranges (represented by the shaded areas). LDD = the least detectable dose.*

erroneous diagnosis. If necessary, very high TSH may be quantified by measuring appropriately diluted samples.

Another potential impediment to the routine use of IRMAs is the method of calculating the standard curve and data reduction. Methods for computing RIAs, such as log-logit used in our laboratory, are not reliable for IRMAs. Nonetheless, appropriate computerization enables rapid analysis while also incorporating quality control features. In the future, the IRMA technique for measuring TSH will replace conventional RIAs for all circumstances of use in the clinical radioligand laboratory. IRMAs have a superior capacity for differentiating abnormal from normal values at no greater cost or technical burden.

The impact of IRMAs on clinical practice has yet to be ascertained, but they have the potential to alter the approach to biochemical interpretation of thyroid dysfunction. A number of advantages have already been uncovered. First, it is possible to detect situations of suppressed TSH secretion with a single blood sample. IRMAs may be more sensitive in identifying hyperthyroidism than is a single measurement of either the FTI, $T_3$, or the free thyroid hormones (1). Of course, a low TSH cannot, and should not, be interpreted in isolation: it could be associated with high thyroid hormone levels, normal levels (ie, autonomous thyroid function), or with low levels as in central hypothyroidism (9) and nonthyroidal illness (10). Second, IRMAs will likely replace the use of TRH stimulation testing to confirm dubious cases of hyperthyroidism. Nonetheless, in situations where it is necessary to determine the adequacy of suppressive therapy with thyroid hormone TRH testing, using the IRMA rather than the RIA should provide additional information (11). Third, high-sensitivity IRMAs have assisted in elucidating the chronobiology of TSH in health and disease (9,12,13). In healthy subjects, the secretion of TSH follows a diurnal pattern: there is a nocturnal surge and a midday nadir. The secretion happens in a pulsatile rather than a continuous manner (12).

Some investigators have suggested that IRMAs be used as the initial screening test for thyroid dysfunction (1,7). This idea is a radical departure, not only in the testing schedule but also in the philosophy of testing, since this would be a venture into consultative reporting. This new "modus operandi" would entail an initial TSH determination. Wayward results, both above and below a previously defined range, would dictate the need for measurement of serum thyroid hormones and in the process give a biochemical diagnosis. The cutoff limits that proscribe further studies are crucial to the utility of this approach. A narrow limit leads to unnecessary testing and expense, whereas a broad limit may lead to misdiagnosis. It is also not yet certain if there is a "gray" zone of TSH values comprised of an overlap between euthyroid and hyperthyroid samples near the lower limit of the normal range. There was none evident in our studies (Fig 7). However, one rare exception to this is pituitary-dependent hyperthyroidism when the TSH level can be normal (14). Use of the IRMA as a screening procedure has some promise, but it does need further study and the understanding that findings must be judged in the light of clinical opinion.

In conclusion, new IRMAs detect TSH at very low levels and thus readily distinguish hyperthyroidism from euthyroidism. They should replace conventional RIAs for routine use. Their full value and impact in clinical practice has yet to be elucidated.

## References

1. Caldwell G, Kellett HA, Gow SM, et al. A new strategy for thyroid function testing. Lancet 1985;1:1117-9.

2. Ross DS. New sensitive immunoradiometric assays for thyrotropin (editorial). Ann Intern Med 1986;104:718-20.

3. Cobb WE, Lamberton RP, Jackson IMD. Use of a rapid, sensitive immunoradiometric assay for thyrotropin to distinguish normal from hyperthyroid subjects. Clin Chem 1984;30:1558-60.

4. Malter JS, Manotti SE, Knee GR, Goodman DBP, Strauss JF III. Identification of hyperthyroid patients by means of a sensitive assay for thyrotropin. Clin Chem 1985;31:642-4.

5. Rosenfeld L, Blum M. Immunoradiometric (IRMA) assay for thyrotropin (TSH) should replace the RIA method in the clinical laboratory. Clin Chem 1986;32:232-3.

6. Spencer CA, Lai-Rosenfeld AO, Guttler RB, et al. Thyrotropin secretion in thyrotoxic and thyroxine-treated patients: Assessment by a sensitive immunoenzymometric assay. J Clin Endocrinol Metab 1986;63:349-55.

7. Klee GG, Hay ID. Assessment of sensitive thyrotropin assays for an expanded role in thyroid function testing: Proposed criteria for analytic performance and clinical utility. J Clin Endocrinol Metab 1987;64:461-71.

8. Pimputkar MR, Feldkamp CS. Radioimmunoassay kit evaluation and selection. J Nucl Med Tech 1985;13:88-94.

9. Caron PJ, Niemann LK, Rose SR, Nisula BC. Deficient nocturnal surge of thyrotropin in central hypothyroidism. J Clin Endocrinol Metab 1986;62:960-4.

10. Ehrmann DA, Sarne DH, Weinberg M. A suppressed serum thyrotropin measured by a sensitive assay may not be diagnostic of thyrotoxicosis (abstract 604). Proceedings of the Endocrine Society, 69th Annual Meeting 1987:172.

11. Heshmati HM, Izembert M, Daqousset F, Nasser H, Vallee G. Ultrasensitive thyrotropin assay after thyrotropin-releasing hormone injection in treated thyroid cancers with undetectable basal thyrotropin levels (abstract 605). Proceedings of the Endocrine Society, 69th Annual Meeting 1987:172.

12. Brabant G, Ranft U, Ocran K, Hesch RD, von zur Muhlen A. Thyrotropin—an episodically secreted hormone. Acta Endocrinol 1986;112:315-22.

13. Evans PJ, Weeks I, Jones MK, Woodhead JS, Scanlon MF. The circadian variation of thyrotropin in patients with primary thyroidal disease. Clin Endocrinol (Oxf) 1986;24:343-8.

14. Jackson JA, Smigiel M, Greene JF Jr. Hyperthyroidism due to a thyrotropin-secreting pituitary microadenoma. Henry Ford Hosp Med J 1987; 35:198-200.