Laboratory Procedure Manual

Analyte: **Thyroid Stimulating Hormone**

Matrix: **Serum**

Method: **Microparticle Enzyme Immunoassay (MEIA)**

Method No.:

Revised:

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Important Information for Users

Coulston periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

Two laboratories performed this testing during 2001-2002. In order to maintain confidentiality of the participants the quality control summary statistics and graphs were combined to mask the individual analysis dates from the two laboratories. Methods for both labs are included in this release. The method for Lab18 analyte is included in this file. The method for Lab40 is described in a separate file.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
lab18	LBXTSH	Thyroid Stimulating Hormone

1. Summary of Test Principle and Clinical Relevance

IMx Ultrasensitive hTSH II is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of human thyroid stimulating hormone (hTSH) in serum or plasma on the IMx analyzer.

The IMx Ultrasensitive hTSH II assay is based on the MEIA technology. The IMx Ultrasensitive hTSH II reagents and sample are added to the reaction cell in the following sequence:

The probe/electrode assembly delivers the sample and anti-hTSH coated microparticles to the incubation well of the reaction cell. The hTSH binds to the anti-hTSH coated microparticles forming an antibody-antigen complex. An aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles is transferred to the glass fiber matrix. The microparticles bind irreversibly to the glass fiber matrix. The matrix is washed with the wash buffer to remove unbound materials. The Anti-hTSH: alkaline phosphatase conjugated is dispensed onto the matrix and binds with the antibody-antigen complex. The matrix is washed to remove unbound materials. The substrate, 4-methylumbelliferyl phosphate, is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.

hTSH or thyrotropin is a glycoprotein with a molecular weight of approximately 28,000 daltons, synthesized by the basophilic cells (thyrotropes) of the anterior pituitary. hTSH is composed of 2 non-covalently linked subunits designated alpha and beta. Although the alpha subunit of hTSH is common to the luteinizing hormone (LH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (hCG), the beta subunits of these glycoproteins are hormone specific and confer biological as well as immunological specificity. Both alpha and beta subunits are required for biological activity. hTSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxin (T_4) and triiodothyronine (T_3), by interacting with specific receptor on the thyroid cell surface. T_3 and T_4 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development and metabolic and neural activity.

The synthesis and secretion of hTSH is stimulated by thyrotropin-releasing hormone (TRH), the hypothalamic tripeptide, in response to low levels of circulating thyroid hormones. Elevated levels of T_3 and T_4 suppress the production of hTSH via a classic negative feedback mechanism. Recent evidence also indicates that somatostatin and dopamine exert inhibitory control over hTSH release, suggesting that the hypothalamus may provide both inhibitory and stimulatory influence on pituitary hTSH production. Failure at any level of regulation of the hypothalamic-pituitary-thyroid axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of T_4 and/or T_3.

In cases of primary hypothyroidism, T_3 and T_4 levels are low and hTSH levels are significantly elevated. In the case of pituitary dysfunction, either due to intrinsic hypothalamic or pituitary disease; i.e., central hypothyroidism, normal or marginally elevated basal TSH levels are often seen despite significant reduction in T_4 and/or T_3 levels. These inappropriate TSH values are due to a reduction in TSH bioactivity which is frequently observed in such cases. Routine TRH stimulating is advised to confirm the diagnosis in such cases. Secondary hypothyroidism typically results in an impaired hTSH response to TRH, while in tertiary hypothyroidism the hTSH response to TRH may be normal, prolonged or exaggerated. Anomalies do occur, however, which limit the use of TRH response as the sole means of differentiating secondary from tertiary hypothyroidism. Although elevated hTSH levels are nearly always indicative of primary hypothyroidism, some rare clinical situations arise which are the result of an hTSH-secreting pituitary tumor (secondary hyperthyroidism). Such patients would display clinical signs of hyperthyroidism.

Primary hyperthyroidism (e.g., Grave=s Disease, thyroid adenoma or nodular goiter) is associated with high levels of thyroid hormones and depressed or undetectable levels of hTSH. The TRH stimulation test has been used in diagnosis of hyperthyroidism. Hyperthyroid patients show a subnormal response to the TRH test. In addition, large doses of glucocorticoids, somatostatin, dopamine and replacement doses of thyroid hormones reduce or totally blunt the hTSH response to TRH.

Earlier assays for serum TSH lacked the sensitivity to be used as a primary test of thyroid function. Sensitive TSH assays now available, with increased ability to clearly distinguish between euthyroid and hyperthyroid populations, are changing thyroid function testing. Analytical sensitivity, as a means of assessing low concentration accuracy, is being replaced by functional sensitivity. The American Thyroid Association has

formally recommended the use of functional sensitivity as means to quantify the sensitivity of TSH assays. Second generation TSH assays, which discriminate between the hyperthyroid and euthyroid patients exhibit a #20% CV at 0.1 □IU/mL. The sensitivity of the IMx Ultrasensitive hTSH II assay meets these criteria (see SPECIFIC PERFORMANCE CHARACTERISTICS section in the assay package insert). Other thyroid tests (FT_4 estimate, T_4, T-uptake and T_3) combined with the ability to accurately measure low levels of hTSH, improve the efficiency of thyroid diagnosis.

2. Safety Precautions

For in vitro diagnostic use only. The safety and handling precautions and limitations for the reagent pack, calibrators, controls and patient samples are described in your IMx System Operation Manual, Section 8. IMX Wash Buffer (Reagent Bottle 4) may cause mild skin or eye irritation. If this solution comes in contact with skin, eyes, or clothing, rinse immediately with water.

Observe Universal Precautions. Wear gloves, lab coat, and safety glasses at all times. Treat all specimens as potentially positive for HIV and Hepatitis B. Dispose of leftover acid solutions as hazardous wastes. All leftover blood specimens and materials that have been in contact with blood must be autoclaved before disposal.

Material safety data sheets (MSDS) for these chemicals are readily accessible as hard copies in the lab

3. Computerization; Data System Management

a. After a run is complete and any additional corrections by the analyst are made, the result file (containing the patient data as well as the QC data) is electronically transferred to the appropriate analyte-specific subfolder into Access on the Local Area Network (LAN). The analyst also gives a hardcopy of the result file to the reviewing supervisor. After the reviewing supervisor approves the final values for release by checking off the bench QC values and signing the hardcopy, he/she sends an email to the computer support staff that the data has been released to be imported into the NHANES 1999+ database that is located in Microsoft Access; the computer support staff imports the data into the NHANES 1999+ database by using a macro. Data entry is verified by the computer support staff and the supervisor. Data is transmitted electronically weekly to Westat's ISIS computer system and transferred from there to NCHS. Abnormal values are confirmed, and codes for missing data are entered by the analyst and are transmitted as part of the data file to the Westat ISIS computer, and are eventually forwarded to NCHS. Westat also prepares the abnormal report notifications for the NCHS Survey Physician.

b. Files stored on the network automatically backed up nightly by LAN support staff. Backup of the daily data containing all raw data files and result files for each run are the responsibility of the LAN staff.

c. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

Universal precautions apply. Serum (preferred) or plasma (heparin or EDTA) specimens may be used with the IMx Ultrasensitive hTSH II assay. Follow the manufacturers processing instructions for serum or plasma collection tubes. Ensure that complete clot formation has taken place prior to centrifugation. Some patient specimens, especially those receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, fibrin may appear as particulate matter. Specimens should be free of particulate matter before analysis.

If the assay will be performed within 24 hours after collection, the specimen should be stored at 2–8°C. If testing will be delayed more than 24 hours, the specimen should be separated from the clot or red blood cells and stored frozen (–10°C or colder). Specimens stored frozen at –10°C or colder for 12 months did not show performance differences. Specimens must be mixed thoroughly after thawing, by LOW speed vortexing or by

gently inverting, and then centrifuged, to ensure consistency in the results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity should be centrifuged before testing.

Sample Volume: 150 µL of specimen is the minimum volume required to perform the assay.

To obtain the recommended volume requirements for IMx Ultrasensitive hTSH II Calibrators and Controls, hold the bottles vertically and dispense 3 drops into the sample well.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

a. Reagents

IMX Ultrasensitive Htsh Ii Reagent Pack, 100 Tests (No. 4B01.20):

1) 1 bottle (8.1) mL) anti-hTSH (mouse, monoclonal) coated microparticles in Tris buffer with protein stabilizers. Preservative: 0.1% sodium azide.
2) 1 bottle (11 mL) anti-hTSH (goat): alkaline phosphatase conjugated in buffer with protein stabilizers. Minimum concentration: 0.1 µg/mL. Preservative: 0.1% sodium azide.
3) 1 bottle (10 mL) 4-methylumbelliferyl phosphate, 1.2 mM in AMP buffer. Preservative: 0.1% sodium azide.
4) 1 bottle (20.2 mL) Wash Buffer containing surfactant.

b. Calibrators

IMX Ultrasensitive Htsh Ii Calibrators (No. 4b01-01):

The six bottles (4 mL each) of IMx Ultrasensitive hTSH II Calibrators are references against the WHO TSH (80/558). Calibrators contain TSH (recombinant) in TRIS buffer with protein stabilizers to yield the following concentrations:

BOTTLE	TSH CONCENTRATION (mIU/mL)
A	0
B	0.5
C	2
D	10
E	40
F	100

Preservative: sodium azide

c. Controls

IMX Ultrasensitive Htsh Ii Controls (No. 4B01-10):
The three bottles (8 mL each) of IMx Ultrasensitive hTSH II Controls contain TSH (recombinant) in TRIS buffer with protein stabilizers to yield the following concentration ranges:

BOTTLE	ULTRASENSITIVE HTSH II CONCENTRATION □IU/mL	RANGE (mIU/mL)
L	0.25	0.15 to 0.35
M	6	4.5 to 7.5
H	30	21 to 39

Preservative: sodium azide

d. Specimen diluent

IMx Ultrasensitive hTSH II Specimen Diluent (No. 4B01-50):
1 Bottle (10 mL) IMx Ultrasensitive hTSH II Specimen Diluent, Tris buffer with protein stabilizers. Preservative: sodium azide.

e. Test Instrument: Abbott IMx System

7. Calibration and Calibration Verification Procedures

The IMx Ultrasensitive hTSH II assay utilizes a four-parameter logistic curve fit (4PLC) data reduction method to generate a calibration curve. The following are assay-specific checks used to evaluate a calibration curve:

ASSAY PARAMETERS	CALIBRATOR EVALUATION (AVGR)
MIN SPAN F-A	Calibrator F - Calibrator A
MAX SAPN F-A	Calibrator F - Calibrator A
MIN CHECK 1	Calibrator A/Calibrator B
MAX CHECK 1	Calibrator A/Calibrator B
MIN CHECK 5	Calibrator E/Calibrator F
MAX CHECK 5	Calibrator E/Calibrator F

RERR (Rate Error)	RMSE (Root Mean Square Error)
" 20	# 0.5

8. Procedure Operating Instructions; Calculations; Interpretation of Results

a. IMX Ultrasensitive hTSH II Assay Parameters

The IMx Ultrasensitive hTSH II assay parameters, illustrated in the package insert, have been factory set. These parameters can be printed, displayed, and edited according to the procedure in your IMx system Operation Manual, Section 6. Ensure that the assay parameters for IMx Ultrasensitive hTSH II assay in the Assay Module match these parameters or edit accordingly. The assay parameters that cannot be edited are noted with an asterisk (*).

NOTE
Result Unit, assay parameter 48.12, can be edited to A13@ (mIU/mL) and Printer Option, assay parameter 48.60, can only be edited to A0@ or A1@. Editing to another number will result in the displayed code A103 Bad Value in Assay File 12 or 60@, respectively, when the assay run is initiated. For further information on changing concentration units and print options, refer to your IMx System Operation Manual, Section 5.

b. IMX Ultrasensitive hTSH II Procedure

The list of required materials and the procedure to a calibration or MODE 1 Assay can be found in the IMx System Operation Manual, Section 5:

The IMx Ultrasensitive hTSH II assay requires a minimum volume of 200 mL of MEIA No. 2 Diluent Buffer in the buffer bottle in order to properly process an assay run. Before initiating the IMx Ultrasensitive hTSH II assay, visually check that at least 200 mL of MEIA No. 2 Diluent Buffer is present. Do not add diluent buffer to the buffer bottle or switch buffer bottles during an assay run.

c. Dilution Information

Specimens with a hTSH value exceeding 100 µIU/mL, (High Range, assay parameters 48.28), are flagged with the code A>100.@ To quantitate the concentration result, perform the Manual Dilution procedure.

d. Manual Dilution

A manual dilution can be performed by making a dilution of the specimen with the IMx Ultrasensitive hTSH II Specimen Diluent (No. 4B01-50 or the IMx Ultrasensitive hTSH II Calibrator A (0 µIU hTSH/mL, No. 4B01A) before pipetting the sample into the sample well. A 1:10 dilution (e.g., 100 µL sample and 900 □L Specimen Diluent or Calibrator A) is adequate for most samples. The dilution should be performed so that the diluted test results read greater than the sensitivity of the assay (0.02 µIU/mL). To determine the concentration of hTSH in the specimen, multiply the concentration of the diluted sample by the dilution factor.

9. Reportable Range of Results

The normal range calculated from these samples (central 95%) was found to be 0.47 to 5.01 µIU/mL (n-515). Samples from 542 apparently healthy individuals were evaluated with the IMx Ultrasensitive hTSH II assay.

10. Quality Control (QC) Procedures

The minimum control requirement for an IMx Ultrasensitive hTSH II Mode II Assay is one control on each carousel. All levels of control will be processed at least 1 time during each 8-hour shift. In addition to Abbott control material, an outside vendor's QC material is included with each run.

When a new lot of the IMx Ultrasensitive hTSH II is used, perform an assay calibration followed by all levels of controls.

IMx Ultrasensitive hTSH II Control values must be within the range specified in the REAGENTS section of the package insert. If a control value is out of its specified range, the test results are invalid and assay recalibration may be indicated.

The results from the pools are checked after each run. The system is declared "in control" if all three QC results are within 2s limits and the run is accepted. If one of the three QC results is outside the 2s limits then apply rules below and reject if any condition is met - the run is then declared "out of control":

- 1_{3s} Any of the three QC results are outside the 3s limit
- 2_{2s} Two of the three QC results in the run are outside the 2s limit (same side of mean)
- R_{4s} Sequential QC results (either within the run or across runs) are outside the 2s limit on the opposite sides of the mean
- 10_x Ten sequential QC results (across pools and across runs) are on the same side of the mean

A QC program s available and should be used to apply these rules to QC data and generate QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC. The initial limits are established by analyzing pool material in 20 consecutive runs and then are reevaluated quarterly. When necessary, limits are updated to include more runs.

While a study is in progress, electronic copies of the QC results from each run are stored in the analyte-specific folder into Access. The analyst also keeps a hardcopy of the QC results from each run.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

IMx Ultrasensitive hTSH II Control values must be within the range specified in the REAGENTS section of the package insert. If a control value is out of its specified range, the test results are invalid and assay recalibration may be indicated.

If the steps outlined above do not result in the correction of the "out of control" values for QC materials, the supervisor should be consulted for other corrective actions. No analytical results should be reported for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

Suspected hyperthyroidism based on low or undetectable hTSH levels should be confirmed with additional thyroid function testing along with other clinical information. Infrequently, hTSH levels may appear elevated due to no specific protein binding. For diagnostic purposes, the hTSH results should be used in conjunction with other data: e.g. symptoms, results of other thyroid tests (e.g. Free T4), clinical impressions, etc.

Performance of this assay has not been established with neonatal specimens.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the IMx Ultrasensitive hTSH II assay.

13. Reference Ranges (Normal Values)

The normal range calculated from these samples (central 95%) was found to be 0.47 to 5.01 µIU/mL (n-515). Samples from 542 apparently healthy individuals were evaluated with the IMx Ultrasensitive hTSH II assay.

14. Critical Call Results (Panic Values)

For NHANES reporting, any result ≤0.1 will be faxed as "≤0.1" to NCHS Medical Officer, and any result ≥10.0 will be faxed, as well. For a description of the flags that appear in the NOTE column on the test results tape, refer to your IMx System Operation Manual, Section 5.

15. Specimen Storage and Handling during Testing

Specimens should remain at room temperature during testing. Special care must be taken to keep samples out of direct light.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

There are no acceptable alternative methods for performing this test. In case of system failure, store all specimens at –20°C until the system is functioning.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an ASCII text file or Excel file, through electronic mail.

For NHANES 1999+, all data are reported electronically to the Westat ISIS computer and then are transferred to NCHS.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

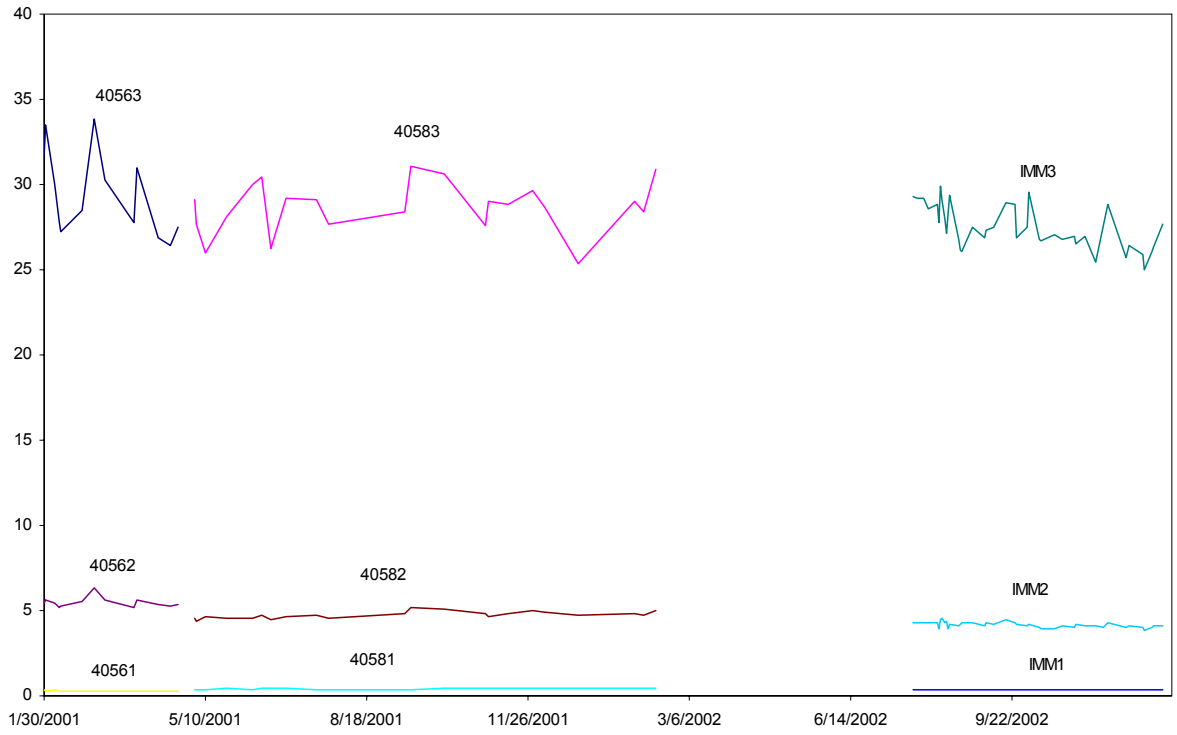
The Microsoft Access database is used to keep records and track specimens for NHANES 1999. All records, including related QA/QC data, are maintained for 10 years after completion of the NHANES study. Only numerical identifiers are used (e.g., case ID numbers).

19. Summary Statistics and QC Graphs

Summary Statistics for Thyroid Stimulating Hormone by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
40561	13	1/30/2001	4/23/2001	0.305	0.011	3.7
40562	13	1/30/2001	4/23/2001	5.471	0.305	5.6
40563	13	1/30/2001	4/23/2001	29.425	2.504	8.5
40581	22	5/3/2001	2/13/2002	0.407	0.021	5.2
40582	22	5/3/2001	2/13/2002	4.738	0.206	4.4
40583	22	5/3/2001	2/13/2002	28.688	1.522	5.3
IMM1	41	7/23/2002	12/24/2002	0.36	0.01	3.5
IMM2	41	7/23/2002	12/24/2002	4.17	0.16	3.8
IMM3	41	7/23/2002	12/24/2002	27.49	1.27	4.6

2001-2002 Thyroid Stimulating Hormone Quality Control



REFERENCES

1. Pierce JG. 1971. The subunits of pituitary thyrotropin. Their relationship to other glycoprotein hormones. Endocrinology. 89:1331-44.

2. Reese-Smith B, Pyle GA, Peterson VB, Hall R. 1977. Interaction of thyrotropin with the human thyrotropin receptor. J Endocrinol. 75:391-400.

3. Sterling K, Lazarus JH. 1977. The thyroid and its control. Annu Rev Physiol. 39:349-71.

4. Patel YC, Alford FP, Burger HG. 1972. The 24-hour plasma thyrotropin profile. Clin Sci. 43:71-7.

5. Morley JE. 1981. Neuroendocrine control of thyrotropin section. Endocr Rev. 2:396-436.

6. Burger HG, Patel YC. 1972. The value of serum thyrotropin measurement in the diagnosis and Management of hypothyroidism. Med J Aust. 2:293-7.

7. Petersen VB, McGregor AM, Belchetz PE, Elkeles RS, Hall R. 1978. The secretion of thyrotrophin with impaired biological activity in patients with hypothalamic-pituitary disease. Clin Endocrinol. 8:397-402.

8. Faglia G, Bitensky L, Pinchera H, Ferrari C, Paracchi A, Beck-Peccoz P, and others. 1979. Thyrotropin secretion in patients with central hypothyroidism: Evidence for reduced biological activity of immunoreactive thyrotropin. J Clin Endocrinol Metab. 48:989-98.

9. Beck-Peccoz P, Amr S, Menezes-Ferreira MM, Faglia G, Weintraub BD. 1985. Decreased receptor binding of biologically inactive thyrotropin in central hypothyroidism. N Engl J Med. 312:1085-90.

10. Kourides IA, Ridgway EC, Weintraub BD, Bigos ST, Gershengorn MC, Maloof F. 1977. Thyrotropin-induced hyperthyroidism; Use of alpha and beta subunit levels to identify patients with pituitary tumors. 1977. J Clin Endocrinol Metab. 45:534-43.

11. Weintraub BD, Gershengorn MC, Kourides IA, Fein H. Inappropriate secretion of thyroid stimulating hormone. Ann Intern Med. 95:339-51.

12. Wehmann RE, Rubenstein HA, Pugeat MM, Nisula BC. 1983. Extended clinical utility of a sensitive and reliable radioimmunoassay of thyroid-stimulating hormone. South Med J. 76:969-76.

13. Lauridsen UB, Deckert T, Friis TH, Kirkegaard C, Hansen JM, Siersback-Nielsen K. 1974. Estimation of serum thyrotropin (TSH) and stimulation with thyrotropin-releasing hormone (TRH) in thyroid diseases. Acta Med Scand. 196:171-6.

14. Jackson IMD. 1982. Thyrotropin-releasing hormone. N Engl J Med. 306:145-55.

15. Spencer CA. 1988. Clinical uses and limitations of rapid TSH assays. Medical Laboratory Products. 1988:17-9.

16. Bayer MF. 1987. Performance criteria for appropriate characterization of A(Highly) sensitive thyrotropin assays. Clin Chem. 33:630-1.

17. Hay ID, Bayer MF, Kaplan MM, Klee GG, Larsen PR, Spencer CA. 1991. American Thyroid Association assessment of current free thyroid hormone and thyrotropin measurements and guidelines for future clinical assays. Clin Chem. 37:2002-8.

18. Hay ID, Klee GG. 1993. Linking medical needs and performance goals: Clinical and laboratory perspectives on thyroid disease. Clin Chem. 39:1519-24

19. Primus FG, Kelley EA., Hansen JH, Goldberg DM. 1988. A Sandwich-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. Clin Chem 1988: 34:261-4.

20. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan Jr AC. 1985. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res. 45:879-85.

21. National Committee for Clinical Laboratory Standards. Evaluation of Precision Performance of Clinical Chemistry Devices, 2nd Ed.; Tentative Guideline. NCCLS Document EP5-T2, March 1992.

22. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Sehn D, Gray D, Nicoloff JT. 1990. Applications of a new chemiluminometric thyrotropin assay to subnormal measurement. J Clin Endocrinol Metab. 70:453-60.