human Thyroid-Stimulating Hormone (hTSH) - Serum
Beckman UniCel DxI Systems

Technical Procedure 3230.T

Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	Oct. 11, 2010	New, renumber from #3218

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human Thyroid-Stimulating Hormone (hTSH) - Serum
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Technical Procedure 3230.T

For In Vitro Diagnostic Use Only

Principle

Intended Use

The Access HYPERsensitive hTSH assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of human thyroid-stimulating hormone (thyrotropin, hTSH) levels in human serum and plasma using the Access Immunoassay Systems. This assay is capable of providing 3rd generation (HYPERsensitive hTSH) results.

Summary and Explanation

Human thyroid-stimulating hormone (hTSH) is one of several glycoprotein hormones consisting of two noncovalently bound peptide chains: an α-chain, which is nearly identical in all, and a ß-chain, which is responsible for immunological and biological specificity. These similarities result in varying degrees of cross-reactivity of different antisera. The Access HYPERsensitive hTSH assay is a solid-phase immunoenzymatic assay with virtually no cross-reactivity to other peptide hormones. The sensitivity and specificity of this test enables better discrimination between hyperthyroid and euthyroid patients.

Human TSH released from the anterior pituitary is the principal regulator of thyroid function. The secretion of thyrotropin-releasing hormone (TRH or TRF) from the hypothalamus controls the secretion of hTSH. This system, in turn, regulates the release of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). A negative feedback mechanism exists which is sensitive to the circulating concentrations of thyroid hormones, T3 and T4, monitored by the hypothalamus. Collectively, this system is referred to as the hypothalamo-hypophyseal-thyroid axis. Any alteration in the function of this axis can influence the levels of T4 and T3 in circulation.(1,2)

Human TSH affects a large number of metabolic processes in the thyroid gland by binding its cellular membrane receptor and activating adenylate cyclase. Adenylate cyclase, in turn, facilitates the production of cyclic AMP, hTSH's "second messenger," which elicits a cascade of metabolic effects intracellularly. This stimulation results in increased synthesis and release of T3 and T4, and maintenance of the physical and functional integrity of the thyroid gland.(3)

The principal clinical use for hTSH measurement is for the assessment of thyroid status. In patients with intact hypothalamic-pituitary function, hTSH is measured to: 1) exclude hypothyroidism (elevated levels of hTSH) or hyperthyroidism (depressed or nondetectable levels of hTSH); 2) monitor T4 replacement treatment in primary hypothyroidism or antithyroid treatment in hyperthyroidism; 3) follow T4 suppression of the trophic influence of hTSH in "cold nodules" and non-toxic goiter; and 4) assess the response to TRH stimulation testing. As more sensitive and precise methods become available, hTSH measurements are also increasingly used to identify subclinical or latent hypothyroidism or hyperthyroidism.(4,5)

Human TSH was originally quantitated using bioassay systems, such as the McKenzie mouse bioassay. However these methods exhibited insufficient sensitivity and required the concentration of large volumes of serum.(6) The preparation of antiserum to hTSH by Utiger et al., led to the development of the radioimmunoassay technique for measuring hTSH.(7,8) The sensitivity of the assay and the small serum sample requirement gave the radioimmunoassay method immediate appeal. Further gains in sensitivity were achieved with chemiluminescent technology.

The HYPERsensitive hTSH assay exhibits performance consistent with the definition of "third generation" functional sensitivity (0.01–0.02 µIU/mL [mIU/L] with an interassay %CV = 20%) for hTSH measurement.(9,10)

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Methodology

The Access HYPERsensitive hTSH assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel with goat anti-hTSH-alkaline phosphatase conjugate, buffered protein solution, and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. (Goat anti-mouse antibody is used to immobilize the mouse anti-hTSH antibody.) The hTSH binds to the immobilized monoclonal anti-hTSH on the solid phase while the goat anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the hTSH. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of human thyroid-stimulating hormone in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Acceptable Sample Containers

13 x 75 SST and Red Top BD tubes
SST and Red Top BD microtainers
Optimum volume: 0.5 mL, Minimum volume: 0.3 mL

Specimen Collection and Preparation

Type of Sample

1. Serum (**Red Top & SST**) is the recommended sample. **Whole blood** and **urine** samples are **not recommended** for use as a sample.
2. Observe the following recommendations for handling, processing, and storing blood samples:(12)
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately. **Each test uses 110 µL of sample**.
   - Store samples tightly stoppered at room temperature (15°C to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2°C to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - **Thaw samples only once**.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer’s recommendations for centrifugation.

Reagents

Access HYPERsensitive hTSH Reagent Pack

Cat. No. 33820: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2°C to 10°C.
- Refrigerate at 2° to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2°C to 10°C.
- Stable at 2°C to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

- All antisera are polyclonal unless otherwise indicated.

R1a	Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-hTSH complexes suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b	TRIS buffered saline with surfactant, BSA, protein (murine, goat) and < 0.1% sodium azide, and 0.1% ProClin** 300.
R1c	Goat anti-hTSH-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA, protein (goat), < 0.1% sodium azide, and 0.1% ProClin** 300.

Warnings and precautions

- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(11)
- Xi. Irritant: 0.1% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The *Safety Data Sheet* (SDS) is available online.

Access HYPERsensitive hTSH Calibrators

Catalog Reorder No. 33825: S0-S5, 4.0 mL/vial
Provided at zero and approximately 0.1, 0.5, 4.0, 10.0, and 100.0 µIU/mL (mIU/L).

- Provided ready to use.
- Store upright and refrigerate at 2°C to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2°C to 10°C.
- Signs of possible deterioration are control values out of range.
- No matrix effects are seen with non-human-serum-based calibrators.
- Refer to calibration card for exact concentrations.

1. S0: Buffered bovine serum albumin (BSA) matrix with surfactant, < 0.1% sodium azide, and 0.5% ProClin** 300. Contains 0.0 µIU/mL (mIU/L) hTSH.
2. S1, S2, S3, S4, S5: Approximately 0.1, 0.5, 4.0, 10.0, and 100.0 µIU/mL (mIU/L) hTSH, respectively, in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.5% ProClin 300.
3. Calibration Cards: 2

- Calibration Cards: One calibration card is provided for the Access HYPERsensitive hTSH assay.

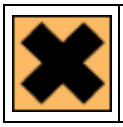
Traceability

The measurand (analyte) in the Access HYPERsensitive hTSH Calibrators is traceable by comparison with a set of primary reference calibrators standardized to the WHO 2nd International Reference Preparation 80/558.(23) Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and precautions

- The antigen used in the preparation of the reagent is derived from human pituitary extracts. Handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.(22).
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(11)
- Xi. Irritant: 0.5% ProClin 300



R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The *Safety Data Sheet* (SDS) is available online.

Access Substrate

Catalog Reorder No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

Condition	Storage	Stability
Unopened	2°C to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15°C to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	Internal substrate supply position	Maximum 14 days

1. R2 Access Substrate: 4 x 130 mL.
   - Lumi-Phos* 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Warnings and precautions

- Substrate is sensitive to air exposure. Keep tightly closed at all times. **Do not pool bottles of substrate**.

Access Wash Buffer II

UniCel DxI Wash Buffer II, Cat. No. A16793: 1 x 10 L

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15°C to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

1. R3 Wash Buffer II: 1 x 10 L.
   - TRIS buffered saline, surfactant, < 0.1% sodium azide, and 0.1% ProClin* 300.

Warnings and precautions

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(11)
- ProClin** 300 is a potential skin sensitizer. Avoid spilling or splashing this reagent on skin or clothing. In case of contact with the reagent, flush thoroughly with soap and water.

Access Sample Diluent A Pack

Cat. No. A79783: 2 diluent packs, 32.9 mL/pack

- Provided ready to use. The Access Sample Diluent A is intended for use with Access assays to dilute patient samples containing analyte concentrations greater than the analyte-specific S5 calibrator.
- Store upright and refrigerate at 2°C to 10°C.
- Refrigerate at 2° to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2°C to 10°C.
- Stable at 2°C to 10°C for 56 days after initial use of each well.
- Signs of possible deterioration are a broken elastomeric layer on the pack.
- If the reagent pack is damaged (i.e., broken elastomer) or if there is evidence of microbial contamination or excessive turbidity in the diluent, discard the pack.

R1a - R1e	Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin** 300.

Warnings and precautions

- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(11)
- Xi. Irritant: 0.5% ProClin 300

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The*Safety Data Sheet* (SDS) is available online.

Equipment

This test is performed on the Beckman UniCel DxI systems; Beckman-Coulter , Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the Beckman UniCel DxI systems *Instructions for Use* manual, *Reference Manual* and/or *Help System* for detailed instructions.

Calibration

An active calibration curve is required for all tests. For the Access HYPERsensitive hTSH assay, calibration is required every 28 days. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual, *Reference Manual* and/or *Help System* for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibrators are assayed in duplicate.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access” format rather than a “batch” format, quality control materials should be included in each 24-hour time period.(13)

At least three levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent lot, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxI *Instructions for Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the operator based on work load and work flow.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the *Control IFUs* folder on the S drive (*S:\APS\ClinLab\PoliciesandProcedures\1000-8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\Control IFUs*). Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on the UniCel DxI 800 Reagent Log sheets. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for information about reviewing quality control results.

Quality Control Material

Control	Storage
MAS OmniImmune 1	until the expiration date at -20°C or colder /30 days at +2°C to +8°C
MAS OmniImmune 2	until the expiration date at -20°C or colder /30 days at +2°C to +8°C
MAS OmniImmune 3	until the expiration date at -20°C or colder /30 days at +2°C to +8°C

Controls are received frozen and stored at –15°C to –20°C.
Bottles of controls in use are thawed and stored at +2°C to +8°C and are good for 30 days.

Thaw controls at room temperature (18° to 25°C) on a rocker until liquid and then immediately store at 2° to 8°C. Thoroughly mix the control bottles before each use by mixing on a rocker for five minutes. Use immediately and return bottles to 2° to 8°C after use.

Testing Procedure

All patient testing will be done by the Access HYPERsensitive hTSH methodology and calibration.

Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for information on managing samples, configuring tests, requesting tests, and reviewing test results.

1. If necessary, load the reagent onto the system. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions. Date, initial cartridge and document in reagent log before loading each new cartridge.
2. After reagent load is completed, calibration may be required. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.
3. Program samples and controls for analysis. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.
4. For assaying samples containing hTSH concentrations up to the concentration of the Access hTSH S5 calibrator (100 µIU/mL), select **hTSH** as the test name.

5. Select **dTSH** as the test name for assaying samples containing hTSH concentrations greater than 100 µIU/mL. The same reagent pack is used for both assays.
6. After loading samples and controls onto the system, follow the protocols for system operation. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.

Procedural Comments

1. Refer to the UniCel DxI *Instructions for Use* manual, *Reference Manual* and/or *Help System* for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. **Do not invert open (punctured) packs**. Document lot number in reagent log, date and initial pack.
3. The HYPERsensitive hTSH assay uses **one hundred ten (110) µL of sample** for each determination **hTSH** in addition to the sample container and system dead volumes. Use **sixty-six (66) µL** of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature (test name: **dTSH**). Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. Serum (**Red Top & SST**) is the default sample type.
5. The system default unit of measure for sample results is µIU/mL. To change sample reporting units to the International System of Units (SI units), mIU/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply µIU/mL by multiplication factor 1.

Reporting Results

Patient Results

Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for complete instructions on reviewing sample results.

Expected Values

1. Human TSH concentrations were measured in 217 human serum samples from apparently healthy male and female subjects (normal thyroid profiles) using the Access HYPERsensitive hTSH assay. The non-parametric range of hTSH concentrations was 0.34–5.60 µIU/mL (mIU/L).
2. In the study described above human TSH values < 0.34 µIU/mL (mIU/L) would be indicative of hyperthyroidism, while values > 5.60 µIU/mL (mIU/L) would indicate hypothyroidism. The evaluation of thyroid status should not depend on results from a single test. Complete thyroid status evaluation should include other thyroid function tests, including evaluation of thyroid autoantibodies (useful in the diagnosis of autoimmune thyroiditis), and the physician's clinical evaluation.
3. Reference interval from a normal study determined at UCDMC by the Access HYPERsensitive hTSH assay: 0.35 - 3.30 µIU/mL
4. Reported Reference Intervals using the Access HYPERsensitive hTSH assay:

TSH Range for Adults (>18 yr)

Clinical Condition	(µIU/mL)
Euthyroid	**0.35 – 3.30**
Hyperthyroid	**less than 0.35**
Hypothyroid	**greater than 5.60**

5. Pediatric TSH Reference Intervals

Age	Gender	Interval
1 day to 1 month	male	0.70 to 9.80 µIU/mL
1 day to 1 month	female	1.50 to 6.50 µIU/mL
1 month to 2 years	male	0.70 to 5.90 µIU/mL
1 month to 2 years	female	1.00 to 5.70 µIU/mL
2 years to 18 years	male/female	0.60 to 4.40 µIU/mL

Pediatric reference intervals were obtained from laboratories using the same test methodology.
No in-house studies were performed. This information should serve as a general guideline.

6. Reflex Testing

When a TSH is ordered with a Free T4 reflex, the Free T4 will be reflexed when the TSH result is below the lower limit of the reference interval for each specific age and gender group or above the upper limit of the reference interval for each specific age and gender group.

Limitations of the Procedure

1. Samples can be measured within the reportable range of the functional (clinical) sensitivity limit to the highest calibrator value (0.02 to 100.0 µIU/mL).
   - If a sample contains less than the functional sensitivity for the assay, report the results as < 0.02 µIU/mL. Dose responses of 0.01–0.02 µIU/mL with interassay (between run) CVs of ≤ 20% are considered to demonstrate "third generation" functional sensitivity performance.(9,10)
   - If a sample contains more than the stated value of the highest Access hTSH Calibrator (S5) (>100.00 µIU/mL), the Onboard Dilution Feature will be used with assay test **dTSH**.

   Onboard Dilution Feature for use on UniCel DxI

   Samples containing hTSH concentrations greater than the concentration of the Access hTSH S5 calibrator can be processed using the DxI onboard dilution feature. The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 4 volumes of Access Sample Diluent A from the UniCel DxI Access Immunoassay Systems Sample Diluent A Pack (**Cat. No. A79783**) allowing samples to be quantitated up to approximately 500 µIU/mL.

Test Name	Reportable Range (µIU/mL)	Sample Volume Required)
dTSH	85 to ~500	66 µL

   Note: The system reports the results adjusted for the dilution. Any neat sample reading < 85 µIU/mL in the **dTSH** assay should be retested in the **hTSH** assay.
   - For short samples not enough for dilution, use the regular 0.5 mL cup rack without flexible volume.
   - Samples with sufficient volume requiring a dilution, but front loaded using the non-flexible volume rack, must be reloaded for the on-board dilution.
   - Short samples QNS for dilution will be reported as **>100.00 uIU/mL, QNS for dilution**.
2. **DO NOT** reuse small sample volumes that have been resident on the analyzer for more than 1 hour.
3. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.(14,15)

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

4. The hTSH results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
5. Evaluate hTSH levels carefully when the hTSH result is inappropriate for the apparent state of thyroid function.(16,17,18)

Performance Characteristics

Analytical Measurement Range

• Samples can be accurately measured within the functional (clinical) sensitivity limit and the highest calibrator value (approximately 0.02 to 100.0 µIU/mL (mIU/L).

Clinical Reportable Range

Samples can be measured within the reportable range of the functional (clinical) sensitivity limit to the highest calibrator value.

• If a sample contains less than the functional sensitivity for the assay, report the results as less than that value. A TSH result less than 0.02 µIU/mL will be reported as **<0.02 µIU/mL**.
• Samples containing more than the stated value of the highest Access HYPERsensitive hTSH Calibrator (S5), >100.00 µIU/mL, will reflex the dilution assay, dTSH, to extend the analytical measurable range from 85 to ~500 µIU/mL. If the dTSH result is greater than 500 µIU/mL, the hTSH result is reported as **> 500 µIU/mL**.
• Short samples > 100.00 µIU/mL will be reported as **>100.00 µIU/mL, QNS for dilution**.

Analytical Sensitivity

The lowest detectable level of hTSH distinguishable from zero (Access HYPERsensitive hTSH Calibrator S0) with 95% confidence is 0.003 µIU/mL (mIU/L). This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

Functional Sensitivity

The literature suggests functional (clinical) sensitivity for hTSH assays is defined in terms of precision. Dose responses of 0.01–0.02 µIU/mL with interassay (between run) CVs of = 20% are considered to demonstrate “third generation” functional sensitivity performance.(9,10) One study using human serum pools, performed with one instrument and during three calibration cycles, with two different reagent pack lots, generating two replicates per assay, over 20 runs, provides the following data on precision:

:

Human Serum Control	Grand Mean (n=40) (µIU/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
Pool A	0.006	40.8	39.6	56.9
Pool B	0.028	12.3	13.0	17.9
Pool C	0.048	10.0	7.3	12.4
Pool D	0.071	3.8	5.0	6.3
Pool E	0.084	4.8	4.4	6.5

The functional sensitivity of 20% between run CV was obtained at 0.015 µIU/mL.

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Methods Comparison

A comparison of hTSH values using the Access HYPERsensitive hTSH assay on the Access Immunoassay system and a commercially available enzyme immunoassay kit gave the following statistical data using Deming calculations:(19)

n	Range of Observations (µIU/mL)	Intercept (µIU/mL)	Slope	Correlation Coefficient (r)
154	0.011 - 79.22	0.208	0.943	0.994
61	0.011 - 1.00	0.028	0.868	0.980

A comparison of 150 paired serum and plasma (heparin) samples using the Access HYPERsensitive hTSH assay on the Access Immunoassay system gave the following statistical data:

n	Range of Observations (µIU/mL)	Intercept (µIU/mL)	Slope	Correlation Coefficient (r)
150	0.006–33.80	-0.067	1.008	1.000

A comparison of hTSH values using the Access HYPERsensitive hTSH assay on the UniCel DxI800 Immunoassay systems (DxI800-602049 & DxI800-602053) and the Centaur XP gave the following statistical data using Deming calculations performed at UCDMC:

Serum (in the range of 0.31 to 118.37 µIU/mL)

Y (DxI800-602049)	= 0.826X + 0.149 µIU/mL
N	= 57
MEAN (DxI800-602049)	= 4.987
MEAN (Centaur XP)	= 5.856
CORRELATION COEFFICIENT (r)	= 0.9989

Serum (in the range of 0.31 to 118.37 µIU/mL)

Y (DxI800-602053)	= 0.784X + 0.164 µIU/mL
N	= 57
MEAN (DxI800-602053)	= 4.754
MEAN (Centaur XP)	= 5.856
CORRELATION COEFFICIENT (r)	= 0.9987

Serum (in the range of 0.25 to 96.96 µIU/mL)

Y (DxI800-602053)	= 0.949X + 0.023 µIU/mL
N	= 57
MEAN (DxI800-602053)	= 4.754
MEAN (DxI800-602049)	= 4.987
CORRELATION COEFFICIENT (r)	= 0.9996

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Dilution Recovery (Linearity)

Multiple dilutions of three samples containing various hTSH levels with Access HYPERsensitive hTSH Calibrator S0 (zero) resulted in the following data:.

Sample 1	Expected Concentration (µIU/mL)	Determined Concentration (µIU/mL)	Recovery (%)
Neat	N/A	0.239	N/A
1/2	0.120	0.119	99.2
1/3.5	0.068	0.063	92.6
1/5	0.048	0.048	100.0
1/7.5	0.032	0.033	103.1
1/10	0.024	0.025	104.2
1/20	0.012	0.011	91.7
		Mean % Recovery	**98.5**

Sample 2	Expected Concentration (µIU/mL)	Determined Concentration (µIU/mL)	Recovery (%)
Neat	N/A	3.33	N/A
1/2	1.66	1.68	101.2
1/3.5	0.95	0.96	101.1
1/5	0.67	0.67	100.0
1/7.5	0.44	0.45	102.3
1/10	0.33	0.31	93.9
1/20	0.17	0.17	100.0
		Mean % Recovery	**99.8**

Sample 3	Expected Concentration (µIU/mL)	Determined Concentration (µIU/mL)	Recovery (%)
Neat	N/A	72.95	N/A
1/2	36.48	34.81	95.4
1/3.5	20.84	20.77	99.6
1/5	14.59	14.30	98.0
1/7.5	9.73	9.68	99.6
1/10	7.30	7.17	98.2
1/20	3.65	3.77	103.3
		Mean % Recovery	**99.0**

Spiking Recovery

Addition of four different levels of hTSH to two patient samples with low hTSH resulted in the following data:

Sample 1	Expected Concentration (µIU/mL)	Determined Concentration (µIU/mL)	Recovery (%)
Neat	N/A	0.43	N/A
Euthyroid	1.43	1.46	102.6
Euthyroid	5.43	5.86	107.9
Hypothyroid	15.43	16.40	106.3
Hypothyroid	80.43	80.36	99.9
		Mean % Recovery	**104.2**

Sample 2	Expected Concentration (µIU/mL)	Determined Concentration (µIU/mL)	Recovery (%)
Neat	N/A	0.99	N/A
1/2	1.99	1.91	95.7
1/3	5.99	6.39	106.6
1/4	15.99	16.10	100.7
1/8	80.99	78.39	96.8
		Mean % Recovery	**100.0**

Imprecision

Precision determined by Beckman

The following study describes the hTSH assay precision. These data are analyzed via analysis of variance (ANOVA).(20,21) One study using commercially available controls, performed with one instrument and during one calibration cycle, generated two assays per day, three replicates per assay, for ten days, provided the following data on precision:.

Human Serum Control	Grand Mean (n=60) (µIU/mL)	Within Run (%CV)	Between Run (%CV)	Total Imprecision (%CV)
Ultralow	0.027	5.83	8.88	10.62
Low	1.00	3.12	3.86	4.96
Medium	8.18	2.53	3.78	4.55
High	28.59	2.49	2.76	3.72

Precision determined at UCDMC

Analyzer	Type of Precision	Control	n	Mean (µIU/mL)	SD	CV (%)
DxI800-602049	Within-run	MAS LiquImmune 1	60	0.223	0.012	5.5
		MAS LiquImmune 3	60	23.940	1.074	4.5
	Between-run	MAS Omni-Immune 1	44	0.149	0.012	8.25
		MAS Omni-Immune 2	44	10.966	0.623	5.68
		MAS Omni-Immune 3	44	20.085	1.131	5.63
DxI800-602053	Within-run	MAS LiquImmune 1	60	0.242	0.016	6.6
		MAS LiquImmune 3	60	21.914	1.333	6.1
	Between-run	MAS Omni-Immune 1	46	0.150	0.013	8.92
		MAS Omni-Immune 2	46	10.585	0.828	7.82
		MAS Omni-Immune 3	46	19.390	1.705	8.79

Analytical Specificity/Interferences

Samples containing 5–9 g/dL (50–90 g/L) albumin, up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triolein, and hemolyzed samples containing up to 500 mg/dL (5 g/L) hemoglobin do not affect the concentration of hTSH assayed.

The following table describes the cross-reactivity of the assay with substances which are similar in structure to hTSH. Crossreactants were spiked into the Access HYPERsensitive hTSH Calibrator S3 with a dose = 4.0 µIU/mL.

Substance	Analyte Added (µIU/mL)	Cross-Reactivity (%)
hLH	3000	0.01
hFSH	1000	0.09
hCG	1,000,000	Not detectable
	(µIU/mL)	
h β-TSH	200	-0.8
h α-TSH	200	5.63

*Lumi-Phos is a trademark of Lumigen, Inc, a subsidiary of Beckman Coulter, Inc.
**ProClin is a trademark of Rohm and Haas Company or of its subsidiaries or affiliates.

References

1. Gornall AG, Luxton AW, Bhavnani BR. Endocrine disorders. Applied Biochemistry of Clinical Disorders 1986: 305-318. Edited by Gornall, A.G. Philadelphia, PA: J. B. Lippincott Co.
2. Watts NB, Keffer JH. The thyroid gland. In Practical endocrine diagnosis 1982: 77-96. Philadelphia, PA: Lea & Febiger.
3. Thyroid Profile Series III. Thyroid stimulating hormone. Rx: RIA for Physicians 1976; 1: 7.
4. White GH. Recent advances in routine thyroid function testing. CRC – Critical Reviews in Clinical Laboratory Sciences 1987; 24: 315-362.
5. Spencer CA. Thyroid status: trends in testing – selective test use cuts cost. Clin Chem 1989; News, 15, No. 11:9-14.
6. Utiger RD. Radioimmunoassay of human plasma thyrotropin. J of Clin Investigation 1965; 44: 8, 1277.
7. Odel WD, Wilbur JF, Paul WE. Radioimmunoassay of thyrotropin in human serum. J of Clin Endocrinology and Metabolism 1965; 23: 1179.
8. Witherspoon LR. August, The laboratory aspects of thyroid function tests: utility and limitations. Endocrinology and Metabolism 1982.
9. Spencer CA, Takeuchi M, Kazarosyan M, MacKenzie F, Beckett GJ, Wilkinson E. Interlaboratory/intermethod differences in functional sensitivity of immunometric assays of Thyrotropin (TSH) and impact on reliability of measurement of subnormal concentrations of TSH. Clin Chem 1995; 41/3, 367-374.
10. Alexander Jr. RL. The diagnostic importance of third-generaton methods for the assay of thyrotropin (TSH). American Clinical Laboratory, 18, December 1994 - January 1995.
11. DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.
12. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
13. Cembrowski GS, Carey RN. Laboratory quality management: QC QA. ASCP Press, Chicago, IL, 1989.
14. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.
15. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.
16. Nahm MH, Hoffmann JW. Heteroantibody: phantom of the immunoassay (editorial). Clin Chem 1990; 36, No. 6: 829.
17. Kricka LJ, et al. Interference by human anti-mouse antibody in two-site immunoassays. Clin Chem 1990; 36, No. 6: 892-894.
18. Wood JM, Gordon DL, Rudinger AN, Brooks MM. Artifactual elevation of thyroid-stimulating hormone. Amer J of Med 1991; 90: 261-262.
19. Cornbleet JP, Gochman N. Incorrect least squares regression coefficients in method-comparison analysis. Clin Chem 1979; 25 (3): 432-438.
20. Approved Guidelines – Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions, C24-A3. June 2006. Clinical Laboratory Standards Institute.
21. Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. Clin Chem 1984; 30: 290-292.
22. HHS Publication, 4th ed., April 1999. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
23. Gaines Das RE, Bristow AF. The second international reference preparation of thyroid-stimulating hormone, human, for immunoassay: calibration by bioassay and immunoassay in an international collaborative study. Journal of Endocrinology 1985; 104: 367-369.
24. Access HYPERsensitive hTSH assay information Sheet, A83792B (3/2010). Beckman Coulter.