Abbott AXSYM SYSTEM

en

Ultrasensitive hTSH II

REF 1L73

48-3458/R5

B1L730

Read Highlighted Changes
Revised June, 2009

Ultrasensitive hTSH II

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	STANDARD CAL A	Standard Calibrator (A-F)
IVD	*In Vitro* Diagnostic Medical Device	CONTROL L	Control Low, Medium, High (L, M, H)
LOT	Lot Number	REAGENT PACK	Reagent Pack
	Expiration Date	SAMPLE CUPS	Sample Cups
2°C 8°C	Store at 2-8°C	MATRIX CELLS	Matrix Cells
15°C 30°C	Store at 15-30°C	REACTION VESSELS	Reaction Vessels
	Consult instructions for use		
	Manufacturer		

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

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WARNING: 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 AxSYM Ultrasensitive hTSH II assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this assay package insert.

NAME

AxSYM Ultrasensitive hTSH II - human Thyroid Stimulating Hormone

INTENDED USE

AxSYM Ultrasensitive hTSH II is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of human thyroid stimulating hormone (hTSH) in human serum or plasma on the AxSYM System. The AxSYM Ultrasensitive hTSH II assay is used as an aid in the assessment of thyroid status.

SUMMARY AND EXPLANATION OF THE TEST

Human thyroid stimulating hormone (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.[1] hTSH is composed of two 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.[1] hTSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), by interacting with a specific receptor on the thyroid cell surface.[2] 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.[3,4] 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.[5] 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.[6] 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 stimulation 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.[7-9] 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 a hTSH-secreting pituitary tumor (secondary hyperthyroidism). Such patients would display clinical signs of hyperthyroidism.[10,11]

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.[12] The TRH stimulation test has been used in diagnosis of hyperthyroidism. Hyperthyroid patients show a subnormal response to the TRH test.[13] In addition, large doses of glucocorticoids, somatostatin, dopamine and replacement doses of thyroid hormones reduce or totally blunt the hTSH response to TRH.[13,14]

Earlier assays for serum TSH lacked the sensitivity to be used as a primary test of thyroid function.[15] 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.[16] The American Thyroid Association has formally recommended the use of functional sensitivity as the means to quantify the sensitivity of TSH assays[17], although analytical sensitivity is still widely used. Second generation TSH assays, which discriminate between the hyperthyroid and euthyroid patients exhibit a ≤ 20% CV at 0.1 µIU/mL.

The sensitivity of the AxSYM Ultrasensitive hTSH II assay meets these criteria (see **SPECIFIC PERFORMANCE CHARACTERISTICS** section in this 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.[18]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM Ultrasensitive hTSH II is based on the Microparticle Enzyme Immunoassay (MEIA) technology.

The AxSYM Ultrasensitive hTSH II reagents and sample are pipetted in the following sequence:

Sampling Center

- Sample and all AxSYM Ultrasensitive hTSH II reagents required for one test are pipetted by the Sampling Probe into various wells of a reaction vessel (RV).
- Sample and Anti-hTSH Coated Microparticles are pipetted into one well of the RV forming an antibody-antigen complex.

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center with the Processing Probe.

Processing Center

- An aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
- The matrix cell is washed with the LDS Wash Buffer.
- The Anti-hTSH: Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds with the antibody-antigen complex.
- The matrix cell is washed to remove unbound materials.
- The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly.

For further information, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

Reagent Pack, 100 Tests

AxSYM Ultrasensitive hTSH II Reagent Pack (1L73-20)*

- 1 Bottle (14.1 mL) Anti-hTSH (Goat): Alkaline Phosphatase Conjugate in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.1 µg/mL. Preservative: sodium azide. (Reagent Bottle 1)
- 1 Bottle (9.0 mL) Anti-hTSH (Mouse, Monoclonal) Coated Microparticles in TRIS buffer with protein (bovine) stabilizers. Preservative: sodium azide. (Reagent Bottle 2)
- 1 Bottle (21.5 mL) LDS Wash Buffer containing surfactant. (Reagent Bottle 3)
- 1 Bottle (47 mL) TRIS Buffer. Preservatives: sodium azide and antimicrobial agents. (Reagent Bottle 4)

* 1L73-20 includes an AxSYM Ultrasensitive hTSH II Reagent Pack (100 tests), reaction vessels (100 each) and matrix cells (100 each).

Calibrators

AxSYM Ultrasensitive hTSH II Standard Calibrators (1L73-01)

6 Bottles (4 mL each) of AxSYM Ultrasensitive hTSH II Standard Calibrators. Performance of the Calibrators is evaluated on Abbott Systems. Standard Calibrator A contains TRIS buffer with protein (bovine) stabilizers. Standard Calibrators B through F contain hTSH (recombinant) in TRIS buffer with protein (bovine) stabilizers to yield the following concentrations:

Bottle	hTSH Concentration (µIU/mL)
STANDARD CAL A	0
STANDARD CAL B	0.5
STANDARD CAL C	2
STANDARD CAL D	10
STANDARD CAL E	40
STANDARD CAL F	100

Preservative: sodium azide.

The calibrators are manufactured gravimetrically and are referenced to the World Health Organization (WHO) Third International Standard 81/565 for TSH (Thyroid Stimulating Hormone) at each concentration level.

Controls

AxSYM Ultrasensitive hTSH II Controls (1L73-10)

3 Bottles (8 mL each) of AxSYM Ultrasensitive hTSH II Controls contain hTSH (recombinant) in TRIS buffer with protein (bovine) stabilizers to yield the concentration ranges below. The Control values are targeted to nominal concentrations as defined by performance on Abbott Systems.

Bottle	hTSH Concentration (µIU/mL)	Range (µIU/mL)
CONTROL L	0.25	0.15 - 0.35
CONTROL M	6	4.5 - 7.5
CONTROL H	30	21 - 39

Preservative: sodium azide.

The AxSYM Ultrasensitive hTSH II reporting unit default setting is µIU/mL. An alternate unit (mIU/L) may be selected for reporting results (Assay Parameter 45). The conversion factor used by the AxSYM System is 1.

Other Reagents

AxSYM Ultrasensitive hTSH II Specimen Diluent (1L73-50)

SPECIMEN DILUENT 1 Bottle (10 mL) AxSYM Ultrasensitive hTSH II Specimen Diluent, TRIS buffer with protein (bovine) stabilizers. Preservative: sodium azide.

Solution 1 (MUP) (8A47-04)

SOLUTION 1 MUP 4 Bottles (230 mL each) Solution 1 (MUP) containing 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: sodium azide.

Solution 3 (Matrix Cell Wash) (8A81-04)

SOLUTION 3 MATRIX CELL WASH 4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M sodium chloride in TRIS Buffer. Preservatives: sodium azide and antimicrobial agents.

Solution 4 (Line Diluent) (8A46)

SOLUTION 4 LINE DILUENT 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M phosphate buffer. Preservatives: sodium azide and antimicrobial agent.

AxSYM Probe Cleaning Solution (9A35-05)

PROBE CLEANING SOLUTION 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammoniumhydroxide (TEAH).

WARNINGS AND PRECAUTIONS

- IVD
- **For *In Vitro* Diagnostic Use.**

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

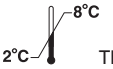
- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens[19]. Biosafety Level 2[20] or other appropriate biosafety practices[21,22] should be used for materials that contain or are suspected of containing infectious agents.
- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.
- This product contains sodium azide; for a specific listing, refer to the REAGENTS section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

Handling Precautions

- AxSYM Ultrasensitive hTSH II reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. Refer to the AxSYM System Operations Manual, Section 9.
- **Do not use Solution 1 (MUP) beyond the expiration date or a maximum of fourteen days on board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.**
- Do not use Reagent Packs beyond the expiration date or a maximum of 336 cumulative hours on board the AxSYM System.
- Do not mix reagents from different reagent packs.

Refer to the AxSYM System Operations Manual Sections 7 and 8 for a more detailed discussion of the safety and handling precautions during system operation.

Storage Instructions

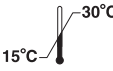


The AxSYM Ultrasensitive hTSH II Reagent Pack must be stored at 2-8°C (do not freeze). The AxSYM Ultrasensitive hTSH II Specimen Diluent, Calibrators and Controls must be stored at 2-8°C. The AxSYM Ultrasensitive hTSH II Reagent Pack, Calibrators and Controls may be used immediately after removing them from the refrigerator. Calibrators and Controls should be returned to 2-8°C storage immediately after use.

Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Ultrasensitive hTSH II Reagent Pack may be on board the AxSYM System for a maximum of 336 cumulative hours; for example, 42 eight hour shifts. Recalibration may be required to obtain maximum on-board reagent stability. More frequent use of controls may be required to monitor reagent performance within the same lot. Refer to the AxSYM System Operations Manual, Sections 2, 5 and Appendices, for further information on tracking onboard time.

Solution 1 (MUP) must be stored at 2-8°C (do not freeze). It may be used immediately after removing it from the refrigerator. MUP may be on board the AxSYM System for a maximum of 14 days. After 14 days, it must be discarded.



The AxSYM Probe Cleaning Solution, Solution 3 (Matrix Cell Wash) and Solution 4 (Line Diluent) must be stored at 15-30°C.

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM Ultrasensitive hTSH II assay file must be installed on the AxSYM System from one of the following software disks, prior to performing Ultrasensitive hTSH II assays:

- 2G37-07, or higher

Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM Ultrasensitive hTSH II Assay Parameters

The default values for the visible assay parameters used for the AxSYM Ultrasensitive hTSH II assay are listed in the following table. Assay parameters that can be edited contain a (>) symbol. These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. However, some parameters that contain a (>) symbol may not be editable if there are no additional options. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter screen. Press PRINT to print the assay parameters.

Assay Parameters
1 Long Assay Name (English): ULTRA_hTSH_II
6 Abbrev Assay Name (English): hTSH_II
11 Assay Number: 208
12 Assay Version: *
13 Calibration Version: *
14 Assay File Revision: *
15 Assay Enabled > ON
17 Assay Type: MEIA
18 Standard Cal Reps > 2
21 Cal A Concentration: 0.000
22 Cal B Concentration: 0.500
23 Cal C Concentration: 2.000
24 Cal D Concentration: 10.000
25 Cal E Concentration: 40.000
26 Cal F Concentration: 100.000
43 Default Dilution Protocol > UNDILUTED
44 Default Calibration Method > Standard Cal
45 Selected Result Concentration Units > ulU/mL
46 Selected Result Decimal Places > 3
64 Max Intercept-Max MUP intercept: *
65 Min Intercept-Min MUP intercept: *
66 Upper limit for NRMSE for low rates: *
67 Upper limit for NRMSE for high rates: *
68 Max Rate-Max rate used to check Min MUP intercept: *
69 Min Rate rate cutoff for NRMSE and Corr. Coef.: *
70 Min correlation coefficient for low rates: *
71 Min correlation coefficient for high rates: *
72 MUP T Delay-Time delay following MUP: *
73 Low Limit-Normal/Therapeutic Range lower limit > 0.000
74 High Limit-Normal/Therapeutic Range upper limit > 0.000
75 Low Extreme Value > 0.000
76 High Extreme Value > 0.000
80 Interpretation Option to use > 0
91 Low Range Undiluted: *
92 High Range Undiluted: *

NOTE: Parameter 45 can be edited to the alternate result unit mIU/L.

NOTE: Parameter 46 cannot be edited.

Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures. For details on Automatic Sample Retest Configuration, refer to the AxSYM System Operations Manual, Section 2, Installation Procedures and Special Requirements.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum (including serum collected in serum separator tubes) or plasma (collected in sodium heparin or tripotassium EDTA) may be used in the AxSYM Ultrasensitive hTSH II assay. Follow the manufacturers processing instructions for serum or plasma collection tubes.
- The AxSYM System does not provide the capability of verifying sample type. It is the responsibility of the operator to verify the correct sample type(s) is(are) used in the AxSYM Ultrasensitive hTSH II assay.
- Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- For optimal results, samples should be free of fibrin, red blood cells or other particulate matter.
- Patient samples should be mixed and centrifuged after any freeze-thaw cycle or to remove red blood cells or particulate matter.
- Multiple freeze-thaw cycles should be avoided. Samples must be mixed thoroughly after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results. Samples have been subjected to three freeze-thaw cycles and showed no performance difference.
- Samples may be stored for up to 7 days at 2-8°C prior to being tested. Serum or plasma should be separated from the clot or red blood cells within 24 hours after collection. If testing will be delayed more than 7 days, samples should be stored frozen at -10°C or colder. Samples stored frozen at -10°C or colder for 12 months showed no performance difference.
- To minimize the effects of evaporation, all samples (patient samples, controls and calibrators) should be tested within 3 hours of being placed on board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for more detailed discussion of onboard sample storage constraints.
- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents.

Sample Volume

The sample volume required to perform a single undiluted Ultrasensitive hTSH II test on the AxSYM System varies depending on the type of sample container used. For sample cups, both ROUTINE and STAT tests require 239 µL. For every additional Ultrasensitive hTSH II test performed (ROUTINE or STAT) from the same container, an additional 189 µL of sample is required.

The sample cup minimum volumes for both STAT and ROUTINE tests are calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is (are) ordered and printed on the Orderlist Report. When using Host Order Query, the Order screen information and Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query Option.

If the assay is configured for Auto Retest/Auto Dilution the additional sample volume needed for the retest will not be displayed on the Order screen at the time the test(s) is(are) ordered. Therefore, the total sample volume should include the additional 189 µL of sample. Refer to the AxSYM System Operations Manual, Section 2, for details on Automatic Sample Retest Configuration.

To obtain the recommended volume requirements for the AxSYM Ultrasensitive hTSH II Calibrators and Controls, hold the bottles **vertically** and dispense 9 drops of each Calibrator or 5 drops of each Control into each respective sample cup.

Refer to the AxSYM System Operations Manual, Section 5, for sample volume requirements in primary or aliquot tubes and Calibrator/control requirements for multiple reagent lots.

AxSYM Ultrasensitive hTSH II PROCEDURE

Materials Provided

- 1L73-20 AxSYM Ultrasensitive hTSH II Reagent Kit, containing:
  AxSYM Ultrasensitive hTSH II **REAGENT PACK**
  100 **REACTION VESSELS**
  100 **MATRIX CELLS**

Materials Required but not Provided

- AxSYM System
- 1L73-10 AxSYM Ultrasensitive hTSH II Controls
- 1L73-01 AxSYM Ultrasensitive hTSH II Standard Calibrators
- 1L73-50 AxSYM Ultrasensitive hTSH II Specimen Diluent
- 8A47-04 **SOLUTION 1 MUP**
- 8A81-04 **SOLUTION 3 MATRIX CELL WASH**
- 8A46 **SOLUTION 4 LINE DILUENT**
- 9A35-05 AxSYM **PROBE CLEANING SOLUTION**
- 8A76-01 **SAMPLE CUPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Order screen

CAUTION:

- When manually dispensing sample into sample cups, verify that dispensing equipment does not introduce cross contamination and delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the System inventory of matrix cells, bulk solutions, and waste levels are acceptable.

The Orderlist Report contains sample placement information and sample cup volume requirements for all ordered tests. It is recommended that this report be referenced when loading samples into sample segments. When using Host Order Query, the Orderlist report is not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query option.

CAUTION: When operating the AxSYM System, always observe the following:

- The System status must be WARMING, PAUSED, READY or STOPPED before adding or removing sample segments, reagent packs or reaction vessels (RVs).
- Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in process. If opened, all processing will stop. Tests in-process will be terminated and must be repeated.
- When testing is completed, it is recommended that samples and the AxSYM Ultrasensitive hTSH II Reagent Pack are removed from the Sampling Center to maximize the on-board reagent pack use. Store at 2-8°C.

SAMPLE DILUTION PROCEDURES

Automated Dilution Protocol

hTSH samples **CANNOT** be diluted automatically on the System.

Manual Dilution Protocol

Patient samples with hTSH concentrations reported as greater than 100 µIU/mL may be diluted using a manual dilution of 1:10. Add 30 µL of the patient sample to 270 µL of the AxSYM Ultrasensitive hTSH II Specimen Diluent (1L73-50). The dilution should be performed so that the diluted test results read greater than the sensitivity of the assay (0.03 µIU/mL). The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

$$\text{Final Sample Concentration} = \text{Reported Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Sample + Volume of Dilution Reagent)}}{\text{Volume of Sample}}$$

QUALITY CONTROL PROCEDURES

Calibration

The AxSYM Ultrasensitive hTSH II assay must be calibrated using a Standard Calibration (6-point) procedure.

Standard Calibration

To perform an AxSYM Ultrasensitive hTSH II Standard Calibration, test the Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of hTSH controls must be tested as a means of evaluating the assay calibration.

Once the AxSYM Ultrasensitive hTSH II calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent pack with a new lot number is used.
- Controls are out of range.

Refer to the AxSYM System Operations Manual Section 6 for:

- Setting up an assay calibration
- When recalibration may be necessary
- Calibration verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendices, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

Quality Control

The recommended control requirement for an AxSYM Ultrasensitive hTSH II assay is a single sample of all hTSH control levels tested once every 24 hours each day of use. Controls may be placed in any position in the Sample Carousel.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

To achieve maximum on-board reagent stability, more frequent use of controls may be required to monitor reagent performance within the same lot.

Ensure that assay control values are within the concentration ranges specified in the package insert. Refer to the **REAGENTS**, **Controls** section of this package insert for AxSYM Ultrasensitive hTSH II Control ranges.

Indications of Instability or Deterioration of Reagents

When an hTSH control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and require retesting. Assay recalibration may be indicated.

Refer to the AxSYM System Operations Manual, Section 10, Subsection: Observed Problems, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

Fluorescence Background Acceptance Criteria

Quality Control with regards to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64 Max Intercept-Max MUP intercept each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, when this error message is obtained.

Refer to the AxSYM System Operations Manual, Section 2, for further information on this parameter.

RESULTS

AxSYM Ultrasensitive hTSH II Assay utilizes a 4-parameter logistic data reduction method (4PLC) to generate a calibration curve. Refer to the AxSYM System Operations Manual, Appendix F, for further information.

Alternate Result Unit

The default result unit for AxSYM Ultrasensitive hTSH II is µIU/mL. When selecting the alternate result unit, mIU/L, the conversion factor used by the AxSYM System is 1.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Section 1.

LIMITATIONS OF THE PROCEDURE

- 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 nonspecific 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 T_4), clinical impressions, etc.
- 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.[23,24] These specimens should not be assayed with the AxSYM Ultrasensitive hTSH II assay.
- Performance of this assay has not been established with neonatal specimens.

Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert.

EXPECTED VALUES

The suggested normal range of the AxSYM Ultrasensitive hTSH II Assay is 0.49-4.67 µIU/mL. This range represents hTSH values (central 95%) obtained by testing serum specimens from 350 apparently healthy individuals. It is recommended that each laboratory establish its own normal range.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The AxSYM Ultrasensitive hTSH II assay is designed to have a precision of ≤ 10% (total CV) at the assay control concentrations. A study based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) document EP5-T2[25] was performed for the AxSYM Ultrasensitive hTSH II assay. A four member panel was assayed, using a single lot of reagents and a single calibration, in replicates of 2 at two separate times per day for twenty days. Panel Member 1 is human serum based, and panel Members 2, 3, and 4 are TRIS buffered protein based. Data from this study are summarized in the following tables. The data in the following tables are representative data; results in individual laboratories may vary from these data.

Panel Member 1

Instrument	Mean Conc. Value (µIU/mL)	n	Within Run SD	Within Run CV(%)	Between Run SD	Between Run CV(%)	Between Day SD	Between Day CV(%)	Total Run SD	Total Run CV(%)
1	0.10	80	0.01	10.78	0.00	4.56	0.01	11.93	0.02	16.72
2	0.07	80	0.01	10.64	0.00	5.11	0.00	1.86	0.01	11.95
3	0.09	80	0.01	10.41	0.01	7.65	0.01	7.34	0.01	14.86
4	0.09	80	0.01	10.73	0.01	6.58	0.00	0.00	0.01	12.58
5	0.09	80	0.01	10.21	0.00	4.07	0.00	3.81	0.01	11.63

Panel Member 2

Instrument	Mean Conc. Value (µIU/mL)	n	Within Run SD	Within Run CV(%)	Between Run SD	Between Run CV(%)	Between Day SD	Between Day CV(%)	Total Run SD	Total Run CV(%)
1	0.26	80	0.02	7.57	0.00	0.93	0.02	6.91	0.03	10.29
2	0.22	80	0.02	7.37	0.00	0.00	0.01	3.00	0.02	7.96
3	0.24	80	0.01	5.89	0.01	2.52	0.01	3.22	0.02	7.17
4	0.23	80	0.02	7.85	0.00	0.00	0.01	4.62	0.02	9.11
5	0.23	80	0.01	4.10	0.00	1.70	0.01	4.38	0.01	6.24

Panel Member 3

Instrument	Mean Conc. Value (µIU/mL)	n	Within Run SD	Within Run CV(%)	Between Run SD	Between Run CV(%)	Between Day SD	Between Day CV(%)	Total Run SD	Total Run CV(%)
1	5.85	80	0.25	4.35	0.13	2.26	0.33	5.73	0.44	7.54
2	5.36	80	0.17	3.08	0.17	3.13	0.10	1.86	0.26	4.77
3	5.66	80	0.20	3.54	0.20	3.61	0.07	1.20	0.29	5.20
4	5.56	80	0.23	4.20	0.04	0.67	0.17	3.03	0.29	5.23
5	6.19	80	0.23	3.75	0.00	0.00	0.16	2.53	0.28	4.52

Panel Member 4

Instrument	Mean Conc. Value (µIU/mL)	n	Within Run SD	Within Run CV(%)	Between Run SD	Between Run CV(%)	Between Day SD	Between Day CV(%)	Total Run SD	Total Run CV(%)
1	28.89	80	1.07	3.71	0.76	2.63	1.79	6.18	2.22	7.67
2	26.50	80	1.20	4.54	0.43	1.61	0.56	2.13	1.40	5.27
3	27.87	80	1.06	3.80	1.25	4.50	0.73	2.60	1.79	6.44
4	27.43	80	1.31	4.78	0.95	3.45	0.31	1.12	1.65	6.00
5	31.40	80	1.00	3.18	0.15	0.48	1.22	3.89	1.58	5.05

Recovery

Known concentrations of hTSH were added to 3 normal human serum samples. The concentration of hTSH was determined using the AxSYM Ultrasensitive hTSH II Assay and the resulting percent recovery was calculated.

Recovery of hTSH

Sample	Endogenous Level (µIU/mL)	hTSH Added (µIU/mL)	Value Obtained (µIU/mL)	Percent Recovery*
1	1.56	4.73	6.43	102.96
2	0.49	4.73	5.37	103.17
3	2.00	4.73	6.33	91.54

Average Percent Recovery: 99.22%

$$*\%\ \text{Recovery} = \frac{\text{hTSH Value Obtained (µIU/mL)} - \text{Endogenous Level (µIU/mL)}}{\text{hTSH Added (µIU/mL)}} \times 100$$

Dilution Linearity

Dilution linearity was investigated by serial dilution of two human serum samples of known hTSH concentrations into the AxSYM Ultrasensitive hTSH II Specimen Diluent.

	Specimen 1			Specimen 2		
Dilution Factor	Expected (µIU/mL)	Observed (µIU/mL)	% Recovery*	Expected (µIU/mL)	Observed (µIU/mL)	% Recovery*
Undiluted	67.16	67.16	100.00	62.32	62.32	100.00
2	33.58	36.74	109.41	31.16	31.35	100.61
4	16.79	18.50	110.18	15.58	16.68	107.06
8	8.40	8.78	104.52	7.79	8.48	108.86
16	4.20	4.22	100.48	3.90	4.16	106.67
32	2.10	2.15	102.38	1.95	2.07	106.15
64	1.05	1.08	102.86	0.97	1.09	112.37
128	0.52	0.53	101.92	0.49	0.52	106.12
256	0.26	0.25	96.15	0.24	0.27	112.50
512	0.13	0.13	100.00	0.12	0.12	100.00
1024	0.07	0.06	85.71	0.06	0.06	100.00

$$*\%\ \text{Recovery} = \frac{\text{Observed}}{\text{Expected}} \times 100$$

Sensitivity

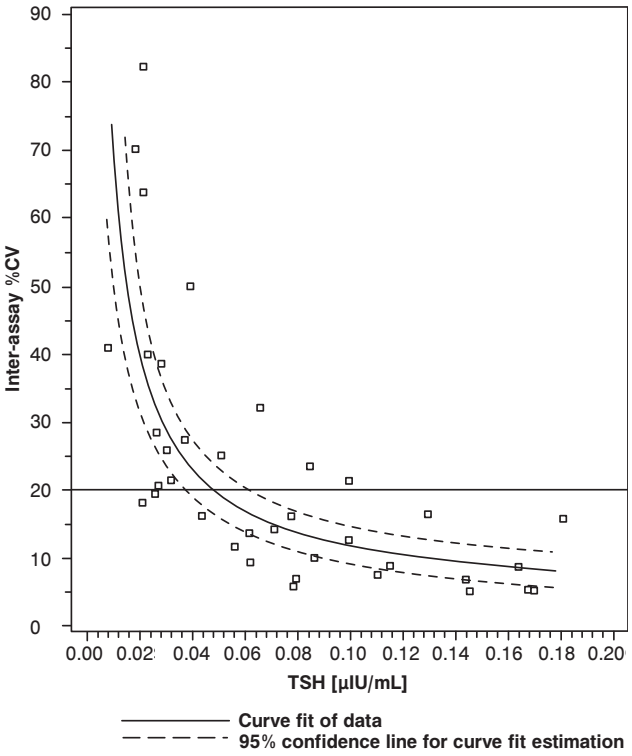
Functional

Functional sensitivity is defined as the concentration of hTSH that can be measured with an inter-assay CV of 20%.[26]

The functional sensitivity of the AxSYM Ultrasensitive hTSH II assay was determined by testing human serum samples ranging from 0.01 to 0.17 µIU hTSH/mL in singlet. Ten assays were performed over a minimum of 10 days. The inter-assay %CV was calculated for each sample and plotted in a precision profile. A curve fit was generated through all the individual data points.

The functional sensitivity of the AxSYM Ultrasensitive hTSH II assay is 0.06 µIU/mL with 95% confidence (the observed mean value was 0.05 µIU/mL) and represents the concentration with a 20% CV from the curve fit.

Functional Sensitivity Precision Profile



Analytical

The analytical sensitivity of the AxSYM Ultrasensitive hTSH II assay was determined to be 0.03 µIU/mL at the 95% quantile (n=63 runs in replicates of 10). Analytical sensitivity is defined as the concentration at two standard deviations from the AxSYM Ultrasensitive hTSH II Calibrator A (0 µIU/mL) and represents the lowest measurable concentration of hTSH that can be distinguished from zero.

Specificity

The specificity of the AxSYM Ultrasensitive hTSH II assay was determined by studying the cross-reactivity of the luteinizing hormone (LH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (hCG). A human serum specimen was spiked with the test hormone and assayed for hTSH activity. The AxSYM Ultrasensitive hTSH II assay displayed no detectable interference with the following hormones in the stated concentrations:

Sample hTSH Concentration (µIU/mL)	Hormone	Hormone Concentration	% Cross-reactivity
2.16	LH	1000 mIU/mL	3.3×10^{-5}
1.13	FSH	1000 mIU/mL	6.3×10^{-4}
1.13	hCG	200,000 mIU/mL	3.1×10^{-8}

Interference

Interference from bilirubin, hemoglobin, and triglycerides was studied in the AxSYM Ultrasensitive hTSH II assay. A human serum specimen was spiked with the interfering substances and assayed for hTSH. The AxSYM Ultrasensitive hTSH II assay demonstrated the interference stated in the following table.

Sample hTSH Concentration (µIU/mL)	Interfering Substances	Interfering Substances Concentration	% Interference
2.19	Bilirubin	15 mg/dL	< 7
1.00	Hemoglobin	1 g/dL	<10
2.19	Triglycerides	1700 mg/dL	< 6

Accuracy By Correlation

The AxSYM Ultrasensitive hTSH II assay is designed to have a slope of 1.0 ± 0.10 and a correlation coefficient (r) of ≥ 0.95 when compared to the IMx Ultrasensitive hTSH II assay.

A study was performed where specimens were tested using the AxSYM Ultrasensitive hTSH II assay and the IMx Ultrasensitive hTSH II assay. Data from this study were analyzed and are summarized in the following table.*

Manufacturer	Number of Observations	Intercept	Slope	Correlation Coefficient
Abbott AxSYM Ultrasensitive hTSH II vs. Abbott IMx Ultrasensitive hTSH II	674	0.26	0.97	0.99

In this evaluation, serum samples tested ranged from 0.06 µIU/mL to 91.87 µIU/mL by AxSYM Ultrasensitive hTSH II.

* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay; therefore, results in individual laboratories may vary from these data.

BIBLIOGRAPHY

1. Pierce JG. The Subunits of Pituitary Thyrotropin. Their Relationship to other Glycoprotein Hormones. *Endocrinology* 1971;89:1331-44.
2. Reese-Smith B, Pyle GA, Petersen VB, Hall R. Interaction of Thyrotropin with the Human Thyrotropin Receptor. *J Endocrinol* 1977;75:391-400.
3. Sterling K, Lazarus JH. The Thyroid and Its Control. *Annu Rev Physiol* 1977;39:349-71.
4. Patel YC, Alford FP, Burger HG. The 24-Hour Plasma Thyrotropin Profile. *Clin Sci* 1972;43:71-7.
5. Morley JE. Neuroendocrine Control of Thyrotropin Secretion. *Endocr Rev* 1981;2:396-436.
6. Burger HG, Patel YC. The Value of Serum Thyrotropin Measurement in the Diagnosis and Management of Hypothyroidism. *Med J Aust* 1972;2:293-7.
7. Petersen VB, McGregor AM, Belchetz PE, Elkeles RS, Hall R. The Secretion of Thyrotrophin with Impaired Biological Activity in Patients with Hypothalamic-Pituitary Disease. *Clin Endocrinol* 1978;8:397-402.
8. Faglia G, Bitensky L, Pinchera H, Ferrari C, Paracchi A, Beck-Peccoz P, *et al*. Thyrotropin Secretion in Patients with Central Hypothyroidism: Evidence for Reduced Biological Activity of Immunoreactive Thyrotropin. *J Clin Endocrinol Metab* 1979;48:989-98.
9. Beck-Peccoz P, Amr S, Menezes-Ferreira MM, Faglia G, Weintraub BD. Decreased Receptor Binding of Biologically Inactive Thyrotropin in Central Hypothyroidism. *N Engl J Med* 1985;312:1085-90.
10. Kourides IA, Ridgway EC, Weintraub BD, Bigos ST, Gershengorn MC, Maloof F. Thyrotropin-Induced Hyperthyroidism: Use of Alpha and Beta Subunit Levels to Identify Patients with Pituitary Tumors. *J Clin Endocrinol Metab* 1977;45:534-43.
11. Weintraub BD, Gershengorn MC, Kourides IA, Fein H. Inappropriate Secretion of Thyroid Stimulating Hormone. *Ann Intern Med* 1981;95:339-51.
12. Wehmann RE, Rubenstein HA, Pugeat MM, Nisula BC. Extended Clinical Utility of a Sensitive and Reliable Radioimmunoassay of Thyroid-Stimulating Hormone. *South Med J* 1983;76:969-76.
13. Lauridsen UB, Deckert T, Friis TH, Kirkegaard C, Hansen JM, Siersbaek-Nielsen K. Estimation of Serum Thyrotropin (TSH) and Stimulation with Thyrotropin-Releasing Hormone (TRH) in Thyroid Diseases. *Acta Med Scand* 1974;196:171-6.
14. Jackson IMD. Thyrotropin-Releasing Hormone. *N Engl J Med* 1982;306:145-55.
15. Spencer CA. Clinical Uses and Limitations of Rapid TSH Assays. *Medical Laboratory Products* 1988:17-9.
16. Bayer MF. Performance Criteria for Appropriate Characterization of "(Highly) Sensitive" Thyrotropin Assays. *Clin Chem* 1987;33:630-1.
17. Hay ID, Bayer MF, Kaplan MM, Klee GG, Larsen PR, and Spencer CA. American Thyroid Association Assessment of Current Free Thyroid Hormone and Thyrotropin Measurements and Guidelines for Future Clinical Assays. *Clin Chem* 1991;37:2002-8.
18. Hay ID, Klee GG. Linking Medical Needs and Performance Goals: Clinical and Laboratory Perspectives on Thyroid Disease. *Clin Chem* 1993;39:1519-24.
19. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
20. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories.* 5th ed. Washington, DC: US Government Printing Office; January 2007.
21. World Health Organization. *Laboratory Biosafety Manual.* 3rd ed. Geneva: World Health Organization; 2004.
22. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition.* CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
23. Primus FJ, Kelley EA, Hansen HJ, Goldenberg DM. "Sandwich"-Type Immunoassay of Carcinoembryonic Antigen in Patients Receiving Murine Monoclonal Antibodies for Diagnosis and Therapy. *Clin Chem* 1988;34:261-4.
24. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan AC Jr. Human Anti-Murine Immunoglobulin Responses in Patients Receiving Monoclonal Antibody Therapy. *Cancer Res* 1985;45:879-85.
25. National Committee for Clinical Laboratory Standards. Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition; Tentative Guideline. NCCLS Document EP5-T2. Wayne, PA: NCCLS, March 1992.
26. Spencer CA, LoPresto JS, Patel A, Guttler RB, *et.al*. Applications of a New Chemiluminometric Thyrotropin Assay to Subnormal Measurement. *J Clin Endocrinol Metab* 1990;70:453-60.

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June 2009