1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access HYPERsensitive hTSH Assay is a two-site immunoenzymatic ("sandwich") assay, for the quantitative determination of human thyroid-stimulating hormone in human serum, using the Access Immunoassay System. 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 serum 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 serum hTSH. Separation in a magnetic field and washing removes materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos® 530, is added to the reaction 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 by means of a stored, multi-point calibration curve.
The major use of the hTSH assay is for the assessment of thyroid status. In patients with intact hypothalamic-pituitary function, hTSH is measured to: 1) exclude hypothyroidism or hyperthyroidism; 2) monitor T4 replacement treatment in primary hypothyroidism or antithyroid treatment in hyperthyroidism; 3) follow T4 suppression in "cold nodules" and non-toxic goiter; 4) assess the response to TRH stimulation testing. hTSH measurements are also used to identify subclinical and latent hypothyroidism or hyperthyroidism.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Germicidal Disposable Wipe when work is finished.
Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.
Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Interferences:

1) No interference from 5-9 g/dL albumin, <10 mg/dL bilirubin or <1800 mg/dL triglycerides.
2) No interference from <500 mg/dL hemoglobin. Hemoglobin does

not affect the concentration of hTSH assayed.

B. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

C. Fasting is not required.

D. A minimum of 0.5 mL serum is needed for the TSH.

E. Sample volume for individual test is 110 µL.

F. Sample is run singly.

4. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation: Beckman Access2 Immunoassay System

B. Materials:

1) Access Immunoassay 1.0 mL Insert Cups (*Cat. #81915*)

2) Access Immunoassay 3.0 mL Sample Container (*Cat. #81914*)

3) Access Immunoassay Reaction Vessels (*Cat. #81901*)

4) Stockwell Scientific Tubes, 13x100mm, polystyrene, (Prod #8570)

5) S/P Plastic Transfer Pipette (*Cat. #P5214-10*)

C. Reagent Preparation:

1) Access HYPERsensitive hTSH Reagent Pack (*Cat. #33820*): 100 determinations, 50 tests/pack. Contains the following components:

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), <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.

a) Provided ready to use.

b) Store upright at 2-10°C.

c) Packs must be refrigerated at 2-10°C for two hours before loading on instrument.

d) Unopened packs are stable until expiration date when stored as directed.

e) After initial use, pack is stable for 28 days at 2-10°C.

f) CAUTION: Sodium azide may react with lead and copper plumbing. On disposal of liquid, flush drain with large volume of water. ProClin is a potential skin sensitizer, in

case of contact with reagent, thoroughly flush with water.

2) Access Substrate (*Cat. #81906*)
   a) Lumi-Phos 530 (buffered solution containing dioxetane Lumigen PPD, flourescer, and surfactant).
   b) Allow substrate to equilibrate, unopened at room temperature for a minimum of 18 hours (maximum 14 days) prior to use.
   c) Unopened substrate is stable until expiration date when stored at 2-10°C
   d) Opened substrate on board in external fluids tray is stable for 14 days.
   e) Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.
3) Access Wash Buffer (*Cat. #81907*).
   a) Tris buffered saline, surfactant, 0.1% sodium azide and 0.1% ProClin 300.
   b) Stable until expiration date when stored at room temperature.

D. Standards Preparation: No preparation required.
   1) Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).

E. Control Material:
   1) Bio-Rad Immunoassay Plus Controls (Levels 1, 2, and 3) (*Cat. #371, 372, 373*).
      a) Reconstitute each vial with 5 mL deionized water using a volumetric pipette. Replace the stopper and let control stand for 15 minutes. Before using, invert vial several times to mix.
      b) Reconstituted control is stable for 7 days when stored at 2-8°C.
      c) At least two levels of control should be analyzed in a 24-hour time period.
      d) Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.
      e) Refer to Quality Control Flow Chart for action decision guidelines.

5. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibrators: Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).
   1) Six levels of calibrator.
   2) Provided ready to use.
   3) Mix contents by gently inverting prior to use.
   4) Stable until expiration date when stored at 2-10°C.
   5) Refer to calibration card enclosed with each set of calibrators for actual concentrations.

B. Calibration:
   1) Calibration is required when a new lot of hTSH reagent is loaded,

when the calibration curve expires (curve stability is 28 days), or when controls are out of range.

2) Refer to Access2 Quick Reference Guide or Access2 "help" icon for detailed instructions on programming a calibration.

6. REPORTABLE RANGE OF RESULTS

A. Analytical Range:

1) 0.01 -The value of the highest calibrator (~100) µIU/mL.

2) A result over range high should be reported as ">100". To obtain a numerical answer, the specimen may be diluted one volume of sample to four volumes of 0.0 Calibrator or Access Sample Diluent A (*Cat. #81908*). After assaying the diluted sample, multiply the printed value by 5 to obtain the reportable answer.

3) Beckman defines sensitivity as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the hTSH determination is 0.003 µIU/mL.

4) 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 $\leq$20% are considered to demonstrate "Third Generation" functional sensitivity performance.

5) CLS will periodically monitor low TSH reproducibility between runs by repeating patient samples. Previously repeated analysis within 1 day of samples with initial values between 0.01 and 0.03 yielded 8 results with no difference and two that differed by 0.01.

6) 0 is not a reportable value. Report results below 0.01 as <0.01.

7. QUALITY CONTROL (QC) PROCEDURES

A. Blind QC Specimens are included in the samples received from NHANES.

B. Bio-Rad Immunoassay Plus Controls levels 1, 2, 3 are assayed prior to running CDC-NHANES samples and after running CDC-NHANES samples.

C. Acceptable Answer:

1) Controls must be within ±2 S.D.

2) Refer to Quality Control Flow Chart for action decisions guidelines.

8. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

9. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Hemolyzed samples with up to 500 mg/dL hemoglobin have no significant interference.
B. <10 mg/dL bilirubin has no significant interference.
C. Lipemia has no significant interference in samples containing equivalent of 1800 mg/dL triglycerides.
D. Samples containing 5-9 g/dL (50-90 g/dL) albumin have no significant interference.
E. This assay has been formulated to minimize the effect of human anti-mouse antibodies or heterophile antibodies which may be present in some patient samples.
F. TSH levels obtained during the first trimester of pregnancy or whenever very high hCG levels are present should be interpreted with caution.

10. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen with dry ice. Specimens are kept frozen at -70°C until ready to analyze. Sample is thawed, mixed well by vortexing, and then transferred to sample cup or sample insert cup on the Access.
Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of Sample I.D. Specimen vial container is placed in -70°C freezer after testing is complete.

11. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Samples will remain in -70°C freezer until instrument is back in operation.

More details see https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/thyrod_g_met_tsh.pdf