

Laboratory Procedure Manual

Analyte: **Thyroid Stimulating Hormone (TSH)**

Matrix: **Serum**

Method: **Roche Cobas 6000 (e601 module)**

As performed by: **University of Minnesota
Advanced Research and Diagnostic Laboratory (ARDL)
1200 Washington Ave S, Suite 175
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Important Information for Users

The Advanced Research and Diagnostic Laboratory (ARDL) periodically refine 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 testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
THYROD_K	LBXTSH1	Thyroid Stimulating Hormone (TSH) (mIU/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Thyroid-stimulating hormone (TSH, thyrotropin) is a glycoprotein formed in specific basophil cells of the anterior pituitary; it is subject to a circadian secretion sequence. The hypophyseal release of TSH is the central regulating mechanism for the biological action of thyroid hormones. TSH has a stimulating action in all stages of thyroid hormone formation and secretion; it also has a proliferative effect.

The determination of TSH serves as the initial test in thyroid diagnostics. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level. Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypothalamus, pituitary and thyroid.

This assay uses a sandwich principle. 50 µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Cobas e601 Application Code: 001

2. SAFETY PRECAUTIONS

Caution: This product is of human and animal origin. Handle as though capable of transmitting infectious disease. Wear appropriate PPE when handling equipment, reagents, and samples.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

ARDL utilizes a highly specialized Laboratory Information System (LIS) (STARLIMS, Abbott Informatics Corporation; Hollywood, FL, 33021-6755) for all lab functions. Major instrument platforms are interfaced directly to the LIS, allowing data to be electronically transferred directly to the main database. The system provides an extensive quality

assurance package and data management tools. Numerous networked computer workstations are used in the laboratory for data management and transmission, and also include software for word and spreadsheet creation and manipulation, statistical analysis, report presentation, and electronic communication. All workstations are user password protected with job specific security access levels and have idle time out functionality. All systems are redundantly backed up on a real time basis.

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

a. **Specimen Type and Requirements:** Use serum that has been separated from the clot within one hour of collection. Serum or plasma (Li-, Na-, NH₄⁺-heparin, K₃-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma) are acceptable. Serum and plasma are stable for seven days at 2-8°C, one month at -20°C, and longer at -70°C. Freeze only once. Specimens must be at room temperature (20-25 °C) prior to assay. Specimens with visible particulates should be centrifuged for 10 minutes at 1500xg before use. This specimen is received frozen and the test is analyzed from NHANES Vial 118.

b. **Specimen Volume:** The required dead volume is 150uL when the specimen is stored in a 2-mL screw cap conical microvial (e.g. Sarstedt #72.664 or Fisher #0554166). Use of larger vials or round/flat-bottomed vials will increase the dead volume requirement. Test sample volume is 50 µL for serum or plasma.**Dead volume is not recoverable if sample is transferred into a sample cup.

c. **Acceptable Specimens/Unacceptable Specimens:** Serum or plasma (Li-, Na-, NH₄⁺-heparin, K₃-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma) are acceptable. Other anticoagulants are not acceptable.

d. **Specimen Stability and Storage:** Separated serum or plasma should be removed from the cells within one hour of collection. Serum and plasma are stable for seven days at 2-8°C, one month at -20°C, and longer at -70°C. Freeze only once. Specimens must be at room temperature (20-25 °C) prior to assay. Specimens with visible particulates should be centrifuged for 10 minutes at 1500xg before use.

e. **Interferences or limitations:**

- Icteric index < 41: no interference.

- Hemolytic index < 1000: no interference.
- Lipemic index < 1500: no interference.
- Biotin < 25 ng/mL: no interference. Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
- No interference was observed from rheumatoid factors up to a concentration of 3250 IU/mL and samples from dialysis patients.
- There is no high-dose hook effect at TSH concentrations up to 1000 µIU/mL.
- In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.
- The presence of autoantibodies may induce high molecular weight complexes (macro-TSH) which may cause unexpected high values of TSH.
- In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

f. **Specimen Handling and Transport:** Mix specimens well, allow clot to fully form (if serum), and centrifuge 10 minutes at 2000 x g before use. Aliquot a minimum of 0.150 mL. Freeze sample until shipment. Ship frozen on dry ice.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

“Not applicable for this procedure.”

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

a. Reagents and Supplies

Roche product # 11731459 122, TSH reagent kit (200 tests):

b. Reagent Preparation (*Reagents are ready to use; no preparation required)

- R1 reagent. Anti-TSH-Ab~biotin (gray cap), 1 bottle, 14 mL: Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

- R2 reagent. Anti-TSH-Ab~Ru(bpy)₂+₃ (black cap), 1 bottle, 12 mL: Monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.
- M. Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- Storage and stability: Keep reagents stored at 2-8°C until use. Unopened, reagents are stable until the expiration date stated on the cassette. The reagents are stable at 2-8°C for 12 weeks once opened, and for 6 weeks refrigerated on the analyzer. Do not freeze. Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

c. Equipment/Instrumentation-

Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN 46250)

- The Millipore Elix Gulfstream Clinical 35 System is designed to meet CLSI Clinical Laboratory Reagent Water (CLRW) standards. Water purification is achieved by reverse osmosis, electrodeionization, bactericidal 254 nm UV lamp and 0.22 µm filtration.

d. Specimens are run in singleton

e. Quality Control

- Roche PreciControl Universal: Ref. # 11731416 160. 4 x 3.0 mL for each level, PreciU1 and PreciU2. To prepare, carefully dissolve the contents of one bottle by adding exactly 3.0 mL of deionized water and allow to stand closed for 30 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the freshly reconstituted controls into empty labeled ControlSet Vials. Attach the supplied labels to the additional bottles. Roche recommends performing only one control procedure per aliquot. The lyophilized controls are stable up to the stated expiration date. Aliquots intended for storage should be frozen immediately at -20 °C. Reconstituted control is stable for three months at -20°C (freeze only once) or at 2-8°C for 3

days. Store calibrators upright in order to prevent the calibrator solution from adhering to the snap-cap.

- Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed at the end of the work day, with change in reagent, after major maintenance, or as needed for troubleshooting.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Roche TSH Calset: Ref. # 04738551 190. 4 x 1.3 mL. A ready-to-use lyophilized equine serum matrix (TSH Cal1) and a human serum matrix (TSH Cal2) with added TSH in two concentration ranges. The Calset can be used with all reagent lots. The exact lot-specific calibrator values are encoded in the barcode as well as printed (or electronically available) on the calibrator barcode sheet. Transfer aliquots of the ready-for-use calibrators into empty CalSet vials. Attach the supplied labels to these vials. Store the aliquots at 2-8°C for later use. Perform only one calibration procedure per aliquot. Stored at 2-8°C, the calibrator is stable for 12 weeks.

Traceability: This method has been standardized against the 2nd IRP WHO Reference Standard 80/558.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:

- After 1 month (28 days) when using the same reagent lot
- After 7 days (when using the same reagent kit on the analyzer)
- As required: e.g. quality control findings outside the defined limits. Monitor control values to determine stability of the current calibration.

If calibration fails perform the following corrective action steps in sequence:

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation, and storage conditions.
- Repeat calibration with new calibrator.
- Repeat calibration with new reagent and new calibrator
- If successful calibration is not achieved, discontinue testing and notify the supervisor.

8. OPERATING PROCEDURE INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

- Instrument Operation:** The Roche/Hitachi Cobas 6000 analyzer series is a fully automated, random-access, software controlled system for immunoassay and photometric analyses intended for qualitative and quantitative in vitro determinations using a wide variety of tests. The Cobas 6000 analyzer series is optimized for workloads using a combination of photometric and ion-selective electrode (ISE) determinations (c501 module), and electrochemiluminescence (ECL) signal in the immunoassay analysis module (e601 module). The ISE system is used in the quantitation of sodium, potassium and chloride. The photometric system can measure colorimetric or immunoturbidimetric reactions utilizing end point or kinetic (rate) absorbance measurements. Test ordering end execution on the Cobas 6000 and data entry in the STARLIMS host computer system may be done manually or these tasks may be executed via a barcode-based bi-directional interface. The Cobas 6000 can utilize both of these two systems simultaneously.
- Professional Judgement:** Check results for error flags and take appropriate corrective action. Investigate alert values and delta checks.
- Result Entry**

STARLIMS test code: TSH

 - Results are reported to two significant digits, as in x.xx mIU/L.
 - Report low results as <0.01 mIU/L.
 - Report high results as >1000 mIU/L.
 - Check results for error flags and take appropriate corrective action.
 - Investigate alert values and delta checks.

9. REPORTABLE RANGE OF RESULTS

Out of Range results: Certain tests have pre-programmed limits that trigger an automatic re-analysis by the COBAS. These limits may be low-end values or high-end values (but within technical range). If the duplicate value is in agreement with the initial value, then the initial value is reported.

Results are reported to two significant digits, as in x.xx mIU/L. Report low results as <0.01 mIU/L.

a. Reportable Range of Test Results: Reportable Range 0.01-1000 mIU/L

Intra-assay %CV (10 within-day replicates at a concentration of 1.55 mIU/L) 1.1%

Intra-assay %CV (10 within-day replicates at a concentration of 8.87 mIU/L) 0.6%

Inter-assay %CV (61 between day replicates at a concentration of 1.596 mIU/L) 2.1%

Inter-assay %CV (56 between day replicates at a concentration of 9.037 mIU/L) 2.9%

Dilutions: The confirmed analytical measurement range of the assay is 0.005-100 mIU/L.

Samples with TSH concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10. The concentration of the diluted sample must be > 10 mIU/L. After manual dilution, multiply the result by the dilution factor.

Reference Ranges:

Roche range:

0.270-4.20 mIU/L

•Advanced Research and Diagnostic Laboratory ranges:

Same as Roche range

Critical Results: None

Analytical Measurement Range: 0.005-100 mIU/L

Reportable Range: 0.01-1000 mIU/L

Limit of Detection (standard 1 + 2 SD): 0.005 mIU/L

10. QUALITY CONTROL (QC) PROCEDURE

•Roche PreciControl Universal: Ref. # 11731416 160. 4 x 3.0 mL for each level, PreciU1 and PreciU2. To prepare, carefully dissolve the contents of one bottle by adding exactly 3.0 mL of deionized water and allow to stand closed for 30 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the freshly reconstituted controls into empty labeled ControlSet Vials. Attach the supplied labels to the additional bottles. Roche recommends performing only one control procedure

per aliquot. The lyophilized controls are stable up to the stated expiration date. Aliquots intended for storage should be frozen immediately at -20 °C. Reconstituted control is stable for three months at -20°C (freeze only once) or at 2-8°C for 3 days. Store calibrators upright in order to prevent the calibrator solution from adhering to the snap-cap.

- Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed at the end of the shift, with change in reagent, after major maintenance, or as needed for troubleshooting.
- The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. Use Roche TSH CalCheck5 (catalog # 05917824 160, Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at 2–8oC. To prepare, reconstitute the contents of Check 1,2,3,4 and 5 each with 1.0 mL MilliQ water using a volumetric pipet; cap and allow to reconstitute for 15 minutes. Mix by gentle inversion. Reconstituted, the product is stable for 4 hours at 20-25oC. Analyze the CalCheck samples in duplicate, programming in the same manner as patient samples. Determine the mean value for each level and compare it to the acceptable range listed on the lot-specific value sheet. The recovery values from the instrument should be within the acceptable limits.
- New Lot Verification: Each new reagent lot must be verified for acceptability before being placed into use. Calibration, quality control, and comparison of at least 5 patient samples on the old and new lots must be performed and found to be within acceptable limits before a new lot can be placed into use.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET

ACCEPTABLE CRITERIA

- If QC values are outside of specified ranges, do the following, in order, until QC is acceptable:
 1. Repeat the analysis using fresh QC material.
 2. Perform a calibration.
 3. Check for system problems.

4. Contact Roche Technical Support for assistance and possible service dispatch.

Phone: 1-800-428-2336; account number: 55042919

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- a. Limit of Detection (standard 1 + 2 SD): 0.005 mIU/L
- b. Analytical Measurement Range: 0.005-100 mIU/L
- c. Interfering Substances and Conditions
 - Icteric index < 41: no interference.
 - Hemolytic index < 1000: no interference.
 - Lipemic index < 1500: no interference.
 - Biotin < 25 ng/mL: no interference. Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
 - No interference was observed from rheumatoid factors up to a concentration of 3250 IU/mL and samples from dialysis patients.
 - There is no high-dose hook effect at TSH concentrations up to 1000 μ IU/mL.
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 - In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

13. REFERENCE RANGES (NORMAL VALUES)

Roche range:

0.270-4.20 mIU/L

Advanced Research and Diagnostic Laboratory range:

- Same as Roche range

14. CRITICAL CALL RESULTS ("PANIC VALUES")

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are frozen at -70°C between sample receipt and analysis on the instrument. Specimens must be at room temperature prior to assay. Specimens are returned to refrigerated or frozen temperature post analysis depending on the study specific requirements.

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

Should the testing system become inoperable, discontinue testing and notify the supervisor. While instrument trouble-shooting or repair occurs; keep specimens at refrigerated or frozen temperature depending on study specific requirements.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

All data is reported electronically via an eFile that is uploaded to the WESTAT secure website within 21 days of receipt of specimens.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Specimen Receipt:

Shipments for NHANES generally will arrive on Tuesdays and/or Wednesdays. These shipments are recorded on the Log of Quality Assurance located in a binder labeled NHANES Shipping Log in the receiving area. The specimen barcode numbers in the boxes are checked against the manifests. The receipt date is written on top of the boxes. The frozen samples (vial 118) are placed in the designated -70°C freezer until analysis. The manifests are filed in a binder labeled NHANES Shipping Manifests located in the receiving area. All labels are removed from the shipping box and the provided airbill is attached for return shipment.

Quality Assurance Log:

A Quality Assurance Specimen Receipt and Specimen Return Log is maintained by laboratory staff. The following parameters are tracked: NHANES shipper I.D., NHANES Container I.D., Vial #, Date Received, Specimen Receipt Conditions, Number of Specimens Received, 2.5% QC Repeats, Total Number of Specimens, 21 Day Due Date, Analysis Date, Date Results Sent, Number of Days For Result Return, Thaw Date

The NHANES spreadsheets are used to set up pending batches for batch accession upload in the Laboratory Information system (STARLIMs). New labels are generated out of the Laboratory Information System (STARLIMs). The new bar-coded labels are attached to a carrier tube. The Cobas analyzer reads the bar-coded label for the sample ID and test information.

Specimen Storage:

The temperatures for all freezers and refrigerators are monitored 24 hours a day/ 7 days a week. If the temperature for any unit falls outside the allowable range, action is taken to resolve the problem. If the temperature cannot be corrected, the contents are moved to a different unit.

Specimen Handling/Specimen Return:

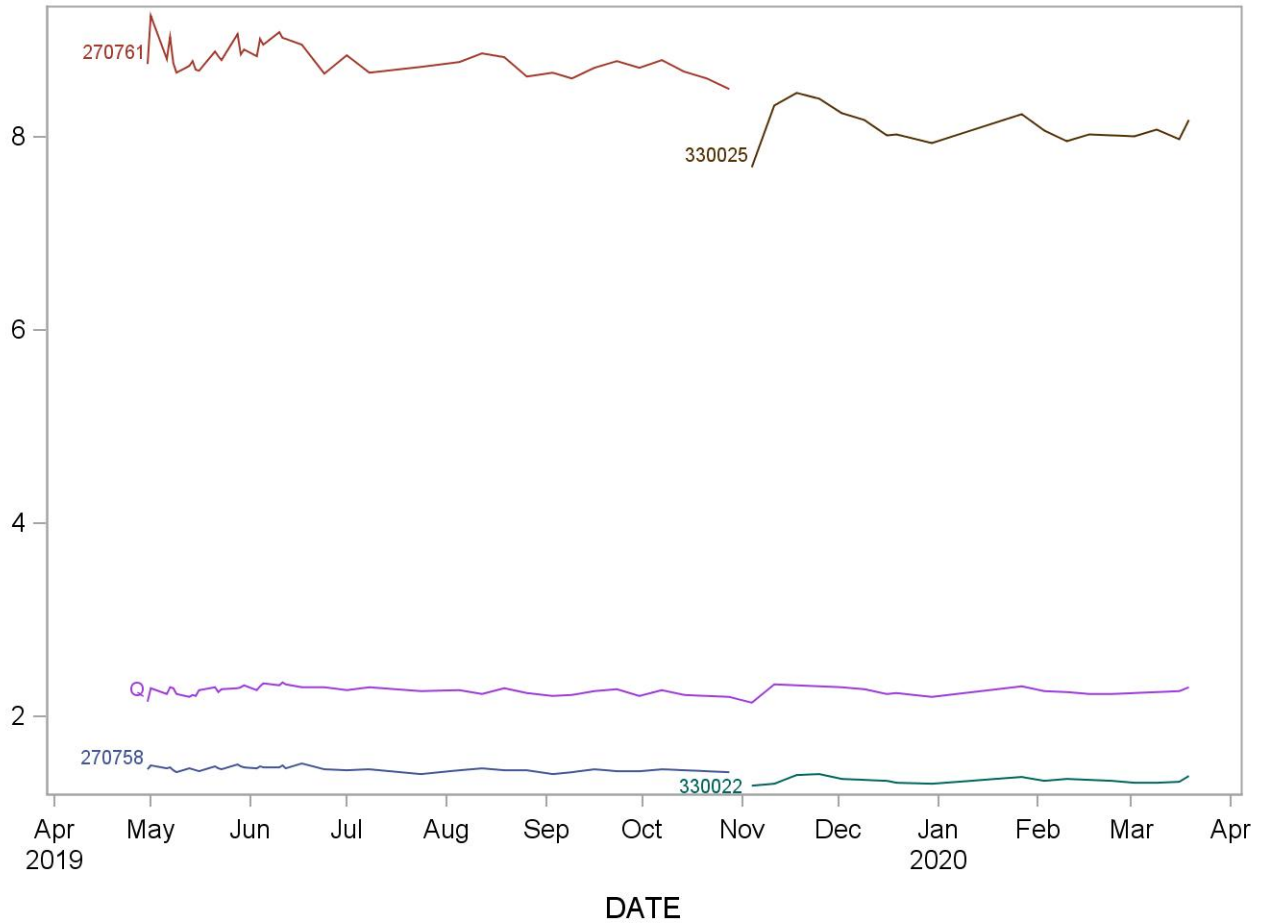
Prior to analysis, the specimens are stored in the designated -70°C freezer. On the day of analysis, the specimens are selected and thawed by the technician operating the COBAS. After analysis and the QC repeats have been run, the specimens are refrozen. After 1 year, the specimen vials that have at least 0.2ml of sample remaining will be shipped to SriSai Biopharmaceuticals in Frederick, MD. These specimens will be shipped on dry ice via Federal Express.

19. SUMMARY STATISTICS AND QC GRAPHS

See following page(s).

**2019-2020 Summary Statistics and QC Chart
LBXTSH1 (Thyroid stimulating hormone (mIU/L))**

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
270761	40	30APR19	28OCT19	8.8160	0.1581	1.8
270758	40	30APR19	28OCT19	1.4520	0.0248	1.7
Q	58	30APR19	19MAR20	2.2633	0.0457	2.0
330025	18	04NOV19	19MAR20	8.1039	0.1858	2.3
330022	18	04NOV19	19MAR20	1.3356	0.0331	2.5



REFERENCES

- Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.
- Roche TSH reagent package insert. 2018-02, V 6.0 English. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
- Roche TSH CalSet package insert. 2017-02, V 9.0 English. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
- Roche PreciControl Universal package insert. 2015-02, V 9.0 English. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
- TSH Calcheck 5 package insert. Version 2017-09, V 5.0 English. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.