

Laboratory Procedure Manual

Analyte: **Parathyroid Hormone**

Matrix: **Serum**

Method: **ECL/Origen-
Electrochemiluminescent**

Method No.:

Revised:

as performed by: *Harborview Medical Center
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Important Information for Users

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

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
I11_c	LBXPT21	Parathyroid Hormone

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Elecsys 1010 analyzer is a fully automatic run-oriented analyzer system for the determination of immunological tests using the ECL/Origen electrochemiluminescent process. All components and reagents for routine analysis are integrated in or on the analyzer. Parathyroid hormone is measured on the Elecsys 1010 using a sandwich principle.

- 1st incubation: 50 µl sample, a biotinylated monoclonal PTH-specific antibody and monoclonal PTH-specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-labeled microparticles, the complex produced is bound to the solid phase via biotin-streptavidin interaction.
- 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. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve. This curve is instrument-specifically generated by a 2-point calibration and a master curve provided via the reagent barcode.
- Total duration of assay is 9 minutes on the Elecsys 1010.

Parathyroid hormone (PTH) is an 84 amino acid peptide produced by the parathyroid gland. Since the PTH molecule undergoes extensive proteolytic modifications, human serum contains both the intact molecule and several fragments. The biologically active N-terminal fragment has a half-life of only a few minutes. The secretory activity of the parathyroid gland can be determined by the selective measurement of the (mainly) intact parathyroid hormone. This Elecsys 1010 method is for the *in vitro* quantitative determination of intact parathyroid hormone in human serum and plasma. Together with vitamin D and calcitonin, PTH brings about the mobilization of calcium and phosphate from the skeletal system and increases the uptake of calcium in the intestine and the excretion of phosphate via the kidneys. Secretion of PTH is inhibited by high calcium concentrations and is promoted by low calcium concentrations. The ratios of intact hormone to peptide fragments may vary from individual to individual as well as between patients with hyperparathyroidism or chronic renal failure. The concentration of metabolically inactive PTH fragments increases in renal failure.

2. SAFETY PRECAUTIONS

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with a 10% bleach solution. Dispose any other potentially contaminated materials in a biohazard bag. Disinfect other non-disposable material at the end of the working day.

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal of personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for all reagents used in the performance of this assay are kept in CNRU in Harborview Hall.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Each shipment of specimens received from the NHANES IV mobile unit arrives with a corresponding transmittal sheet and a Send File (a comma delineated text file) transmitted electronically (labeled boxnum.shp). This file contains the following information:

Send File

Field	Type
Sample ID	XXXXXXXXXX
Slot Number	XXX
Sample Collection Date	mm/dd/yyyy hh:mm:ss
MEC Comment Code	XX

After the testing is completed, the run number, date of analysis, CRP result, CRP comment, CRP analyst, and the CRP 2.5% repeat results are entered into the result file.

Data entry is checked for errors.

After the *H. pylori* testing has also been completed, results generated, and checked, the result file is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files are kept in the laboratory.

Technical support for this system is provided by Westat, Rockville, MD (1-301-294-2036)

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

- A. No special instructions such as fasting or special diets are required.
- B. mL EDTA plasma. Mix sample well before loading into carousel.
- C. Preference should be given to EDTA plasma, as it is stable longer than serum and faster to process.
- D. Plasma: Stable for 2 days at 15–25°C, 3 days at 2–8°C, 6 months at –20°C.
- E. Serum: Stable for 8 hours at 15–25°C, 2 days at 2–8°C, 6 months at –20°C.
- F. The patient samples, calibrators, and controls must be at ambient temperature (20–25°C) before measurement.
- G. Avoid freeze/thaw cycles. Samples may be frozen only once.

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

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

A. Reagents, Standards, and Control Materials

(1) Elecsys PTH kit, Cat. No. 11972103-100 tests

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: streptavidin-coated microparticles, 0.72 mg/mL; binding capacity: 470 ng biotin/mg microparticles; preservative.
- R1 Anti-PTH-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-PTH antibody (mouse) 2.3 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.
- R2 Anti-PTH-Ab~Ru (bpy) 2+3 (black cap), 1 bottle, 10 mL: monoclonal anti-PTH antibody (mouse) labeled with a ruthenium complex 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

- (2) Reagent pack must be stored and kept in an upright position at all times. Store it at 2–8°C when not in use. The pack must be allowed to come to room temperature 40–45 minutes before use. Do not mix manually. Resuspension of microparticles takes place automatically by the Elecsys 1010 before use. Open bottle caps manually before use and keep closed after use. The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read via the reagent bar code. The reagent pack should last 4 weeks when stored alternately in the refrigerator and on the analyzer. Any change in lot number of PTH reagent or PTH cal set will require parallel analysis of at least 100 samples covering the full clinical sample range with the old reagent lot vs. new reagent lot, before put into use for NHANES samples.
- (3) PTH Cal Set. Cat. No. 11972219. Elecsys PTH Cal Set consists of lyophilized human serum with added synthetic PTH in two concentration ranges (approximately 0.05 pg/mL and 4500 pg/mL). The exact lot specific calibrator values are encoded in the bar code and are printed on the calibrator bar code sheet. Carefully dissolve the contents of each bottle by adding volumetrically, 1.0 mL type 1 water to each and allow standing closed for 15 minutes to reconstitute. Mix carefully by gentle inversion, avoiding formation of foam. Transfer the reconstituted calibrators evenly into two sets of the empty, labeled Elecsys snap-cap bottles supplied, making sure they are in the correct bar code labeled bottles. Freeze one set at –20°C for use later. Date both sets of containers with date diluted. Lyophilized calibrators are stable until expiration date. Reconstituted/thawed calibrators are stable 2 weeks at 2–8°C or 3 months at –20°C (freeze once only) or 5 hours at 20–25°C.
- (4) ProCell. Cat No. 1662988. Phosphate buffer 300 mmol/L, tripropylamine 180 mmol/L, detergent < 0.1%, preservative, pH 6.8. Stable up to stated expiration date when stored unopened at 15–25°C. Stable for a total of 3 days on the analyzer when open or 4 weeks on the analyzer when closed. Keep reagent capped when instrument is not in actual use. Unbound substances are removed with ProCell in the measuring cell.
- (5) SysWash. Cat. No. 11930316. Detergent 7.5%. Stable up to stated expiration date when stored 2–8°C. Make up to 4 liters at time as needed. Rinse out the wash bottle located on the instrument and fill to 4L mark BEFORE ADDING 40 mL of the SysWash Concentrate. An approximate 10% concentration of SysWash is desired. Do not go over 10% or testing problems may result.
- (6) Clean Cell. Cat. No. 11662970. KOH 176 mmol/L. Stable up to stated expiration date when stored at 15–25°C. Reconditions the measuring cell.
- (7) Diluent Universal. Cat. No. 03183971. Protein matrix; preservative < 0.1%. Stable up to stated expiration date when stored at 2–8°C. Stable up to 3 months when stored on analyzer at 20–25°C.
- (8) SysClean. Cat. No. 1298500. Sodium hydroxide solution 12%, sodium hypochlorite solution <2%. Stable up to stated expiration date when stored at 2–8°C. Protect from light. Warning: Causes severe burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Remove contaminated clothing immediately. Wear suitable gloves and eye/face protection when using.
- (9) PTH CalCheck. Cat. No. 12144743. A set of three lyophilized calibration verification solutions of known concentration. Reconstitute each with 1 mL of type 1 water before use. Stable unopened at 2–8°C until the expiration date. Stable once reconstituted for 4 hours at room temperature.
- (10) PreciControl Bone. Cat. No. 11972227. PreciControl Bone is used for quality control in the Elecsys 1010. It contains lyophilized control serum based on equine serum in three concentration ranges. The approximate ranges are 60 pg/mL, 205 pg/mL, and 850 pg/mL for PC Bone 1, PC Bone 2, and PC Bone 3, respectively. The exact target values and ranges are given in the bar codes as well as in the enclosed data sheet. Carefully dissolve the contents of each bottle by adding volumetrically, 2.0 mL type 1 water to each and allow standing closed for 15 minutes to reconstitute. Mix carefully by gentle inversion, avoiding formation of foam. Transfer the reconstituted calibrators evenly into two sets of the empty, labeled Elecsys snap-cap bottles supplied (ControlSet Vials), making sure they are in the correct bar code labeled bottles. Freeze one set immediately at –20°C for use later. Date both sets of containers with date diluted. Lyophilized controls are stable until expiration date. Reconstituted/thawed controls are stable 5 days at 2–8°C or 1 month at –20°C (4 freeze/thaw cycles possible) or 8 hours at 20–25°C.

B. Equipment

- (1) Elecsys 1010 Immunoassay analyzer
- (2) Elecsys 1010 assay cups, Cat. No. 1706829
- (3) CalSet Vial, Cat. No. 1776576
- (4) Transfer pipettes
- (5) Sample Cups
- (6) External printer and paper

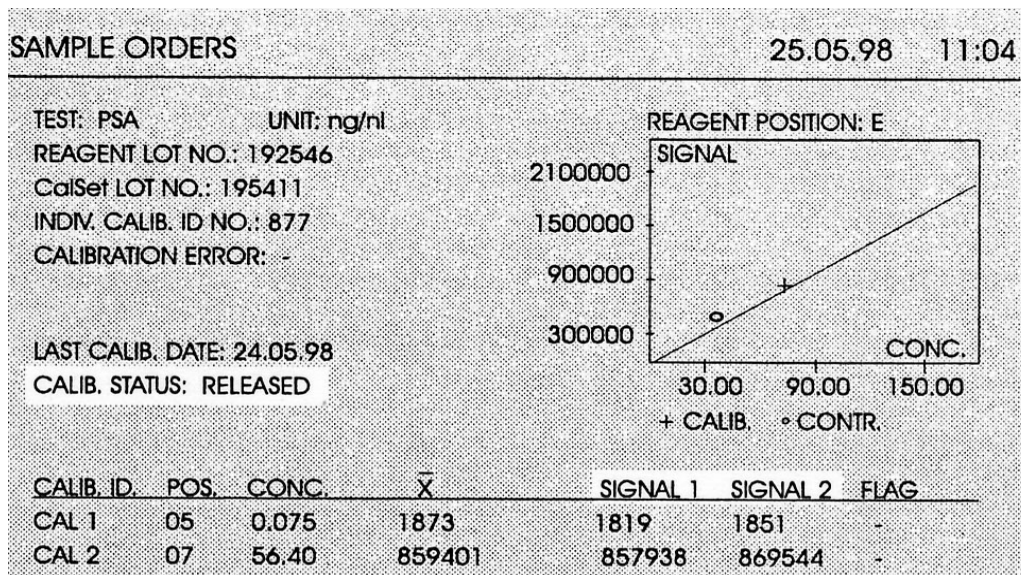
C. Instrumentation

The Elecsys 1010 is a routine and STAT Immunoassay analyzer with a throughput of 58 tests/hour. Bar-coded and non bar-coded samples can be run simultaneously. It can accommodate calibration, control vials, and patient primary sample tubes. Two dimensional bar code cards are used to read assay parameter and control value information into the Elecsys 1010 analyzer computer. In this electrochemiluminescence (ECL) immunoassay, conjugate is ruthenium-based and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of antigen in the sample. The measuring cell is the core of the system. It is sealed in a light-proof and temperature-controlled (28°C) chamber. This measuring cell chamber consists of a working electrode, counter electrode, a magnet, and a photomultiplier. When the reaction mixture (sample and reagent) is placed in the measuring cell, three processes are performed to produce measuring signals.

- (1) Bound/Free Separation: Streptavidin microparticles which are coated with antibody-antibody complexes are uniformly deposited on a defined spot of the working electrode. This sits for a few seconds and ProCell is flushed through the measuring cell washing the microparticles on the working electrode and flushing out excess reagent and sample material.
- (2) ECL Reaction: Voltage is applied to the working electrode to initiate the electrochemiluminescent reaction. The light emission produced is measured by a photomultiplier. These signals are used by the system to calculate results.
- (3) Releasing the Microparticles: Once measurement is complete, the measuring cell is reconditioned with CleanCell and is ready for new measurements.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve



A full calibration is not necessary when using the Elecsys 1010 analyzer. A lot specific master calibration curve is generated by Boehringer Mannheim. The master curve parameters are encoded into each reagent pack two dimensional bar code label for the corresponding lot of reagent. This information is entered into the Elecsys 1010 analyzer when the reagent pack is scanned by the bar code reader. A two-point calibration is required for each new reagent pack, each new reagent lot, every 3 days if the ambient temperature of the laboratory is between 25–32°C , and every 7 days if the temperature is between 20–25°C. For the NHANES study, a calibration curve is done once for every reagent pack per day.

B. Validation

Calibration validation is performed automatically by the instrument software. The calibration values of each calibrator level undergo a stepwise quality criteria check.

The curve is automatically released by the system if all of the following conditions are met:

- (1) All calibrator signals are above the recommended minimum signal level.
- (2) No calibrator values are missing.
- (3) No duplicate errors.

Calibration is questionable and the curve is blocked by the system if any of the following conditions occur:

- (1) One signal value is missing from a calibrator pair.
- (2) One signal value falls below the recommended minimum signal level.
- (3) One calibrator pair exceeds the duplicate limit.

Calibration is failed and the curve is blocked by the system if any of the following occur:

- (1) Two or more signal values are missing from a calibrator pair.
- (2) Two or more signal values are below the recommended minimum signal level.
- (3) Two calibrators are measured with a duplicate error.
- (4) Slope of the calibration is not acceptable.

C. Verification

- (1) Three levels of control are run for each batch of samples. If, within a testing batch, these controls do not conform to specifications as defined in the quality control manual, the entire series is invalidated.
- (2) CNRU uses a set of horse based controls called BONE1, BONE2 and BONE3. These are NOT used by NHANES.
- (3) Each batch should include CNPTHL, CNPTHM and CNPTHH, which are human-based homemade controls.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

- (1) Bring all controls, standards, and patient specimens to room temperature before use (40–45 minutes). All reagents/controls/calibrators are very susceptible to bubble formation. Care must be taken not to shake or mix up the reagents. Reagent packs must be stored upright to prevent the microbeads from sticking to the lid of the reagent.
- (2) All patient samples must be mixed well.

- (3) The instrument will be left on. Turn the dim switch up on the computer screen by pushing the “+” soft key.
- B. Instrument Operation (see operator's manual for more details).
- (1) Inventory Check-press [INVENTORY] soft key located on top left of gray soft keys. Highlight [Inv. Rgts] at bottom of screen with soft key along bottom. Fill water container with 4 L of type 1 water and no more than 40 mL of the SysWash found in the refrigerator. Empty waste container. This must go down the sink in the stool fat hood bench since it may release cyanide gas if it comes into contact with bleach. Check the ProCell and CleanCell volumes of both sets. At each step update the inventory. Highlight [Inv. Assay Cups] at bottom of screen with soft key along bottom. Check the assay cup supply and replace sections as necessary. Cups that have been used will be black. Clean cups are white. Update sections as they are replaced.
 - (2) Load the reagents. Put reagent cartridge into selected position. It is not necessary to open the caps until right before the start of the run. Put the zero bar code tube in position #1 of the outside lower ring. Press the [SCAN] soft key at the top of the instrument and allow Elecsys to scan the reagent pack. It will attempt to scan all positions.
 - (3) Check the “Calibration Status” using the [CALIBRATION] soft key at the left side of the keypad. If a calibration is required load the two calibrators and three controls in the lower outside ring starting with position 1, using only the odd numbers (1, 3, 5, 7, etc) Even positions do not work. Load the zero bar code at the end. Do not load samples with the calibration run. [SCAN] the cal/QC bar code cards if a new lot of calibrator or QC is being used.
 - (4) Open the lids of the ProCell and CleanCell (both sets), reagents, calibrators, and controls.
 - (5) Press the green [START] soft key at the top and allow running. When the calibration is complete, press the soft [CALIBRATION] key and view the results and verify that the calibration is “released”.
 - (6) Check to make sure that the QC results are within the posted ranges. If the calibration and QC are acceptable, recap the calibrators and controls as soon as possible. Patient samples are ready to be run.
 - (7) To run samples, load bar-coded samples onto the lower deck of the sample reagent disk starting in position 1. Load a zero barcode after the last bar-coded sample. Press the [SCAN] button to register the samples. After the scan, a window may appear showing empty bar code positions, verify that the positions are empty, then press [CLOSE]. Next press [ORDERS] and [SAMPLE ORDERS] if this screen is not displayed. Select [PTH] test for each sample by pressing the test soft keys. Press [NEXT] to scroll to the next sample. When complete, press [SAMPLE LOADING] to verify the programming.
 - (8) The instrument automatically calculates all results. After testing is completed, results are printed and review by the technologist.
 - (9) Remove specimens, controls, and reagents. Return them to the refrigerator.
 - (10) Perform scheduled instrument maintenance (daily, weekly, monthly) as outlined on the maintenance log. See the operator's manual for specific instructions.
 - (11) Close the lids for the ProCell and CleanCell, close the instrument lid and dim the screen by pushing the “-“ soft key.
- C. Recording of Data
- (1) Specimen results are entered into the assay specific results table created from the send file corresponding to the specific sample box using Excel software (Microsoft Corporation, Redmond WA). A copy of this table is printed out and checked for accuracy of data entry.
 - (2) Control results are entered to the Assay Specific QC/Levey-Jennings Table using the Excel program. Compliance with the Westgard rules is evaluated. A copy of this table is printed out and checked for accuracy of data entry.

D. Replacement and Periodic Maintenance of Key Components

(1) Daily Maintenance:

None required

(2) Every 2 Weeks:

(a) Clean the Sample/Reagent Probe

(b) Clean the Microparticle Mixer

(c) Clean the Sipper Probe

(d) Perform the System Cleaning Intensive Procedure

(e) Perform the Cell Cleaning Normal Procedure

(3) Every 2 Months:

Clean the Rinse Stations

E. Calculations

Elecsys 1010 automatically calculates the PTH concentration of each sample in pg/mL. For detailed information on how Elecsys calculates results, refer to the Elecsys Operator's Manual.

9. REPORTABLE RANGE OF TEST RESULTS

Linearity: 9.3 to 4300 pg/mL. Results >4300 pg/mL are reported as ">4300 pg/mL.". Results <9.3 are reported as "<9.3 pg/mL".

10. QUALITY CONTROL (QC) PROCEDURES

Three levels of controls are used as quality control for each run. (Optional) Run PreciControl Bone levels 1-3—the results are NOT used by NHANES. Run homemade controls CNPTHL, CNPTHM and CNPTHH per batch. Consult the posted Quality Control chart for current lot numbers and ranges.

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

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- A. Per package insert, criterion is recovery within + 10% of initial value. The assay is unaffected by icterus (bilirubin <65 mg/dL), hemolysis (Hb <1.5 g/dL)—verified up to 0.1 g/dl Sep 2003, lipemia (Intralipid <1500 mg/dL)—verified up to 120 mg/dl Sep 2003 and biotin <50 ng/mL.
- B. No interference was observed from rheumatoid factors up to a concentration of 1500 U/mL. (verified at HMC up to RF of 1025 2/2004)
- C. In patients receiving therapy with high biotin doses (i.e. >5 mg/day) no sample should be taken until at least 8 hours after the last biotin administration.
- D. As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.
- E. No high dose hook effect at PTH concentrations of up to 570,000 pg/mL occurred (verified at HMC 9/2003).

- F. *In vitro* tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.
- G. In rare cases, interference due to extremely high titers of antibodies to ruthenium can occur. Elecsys PTH contains additives to minimize these effects.
- H. Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

13. REFERENCE RANGES (NORMAL VALUES)

The reference range was determined to be 18–74 pg/ml in normal adults by testing 214 NHANES subjects on Sep 2003. 95% of normal subjects fall on this range.

14. CRITICAL CALL RESULTS (PANIC VALUES)

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be maintained at 20–25°C during testing. After testing, the samples are stored at 2–8°C.

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

There are no acceptable alternative methods of analysis. Plasma specimens may be stored at 2–8°C for no longer than 3 days. Do not refreeze samples.

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

Not applicable to this procedure.

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

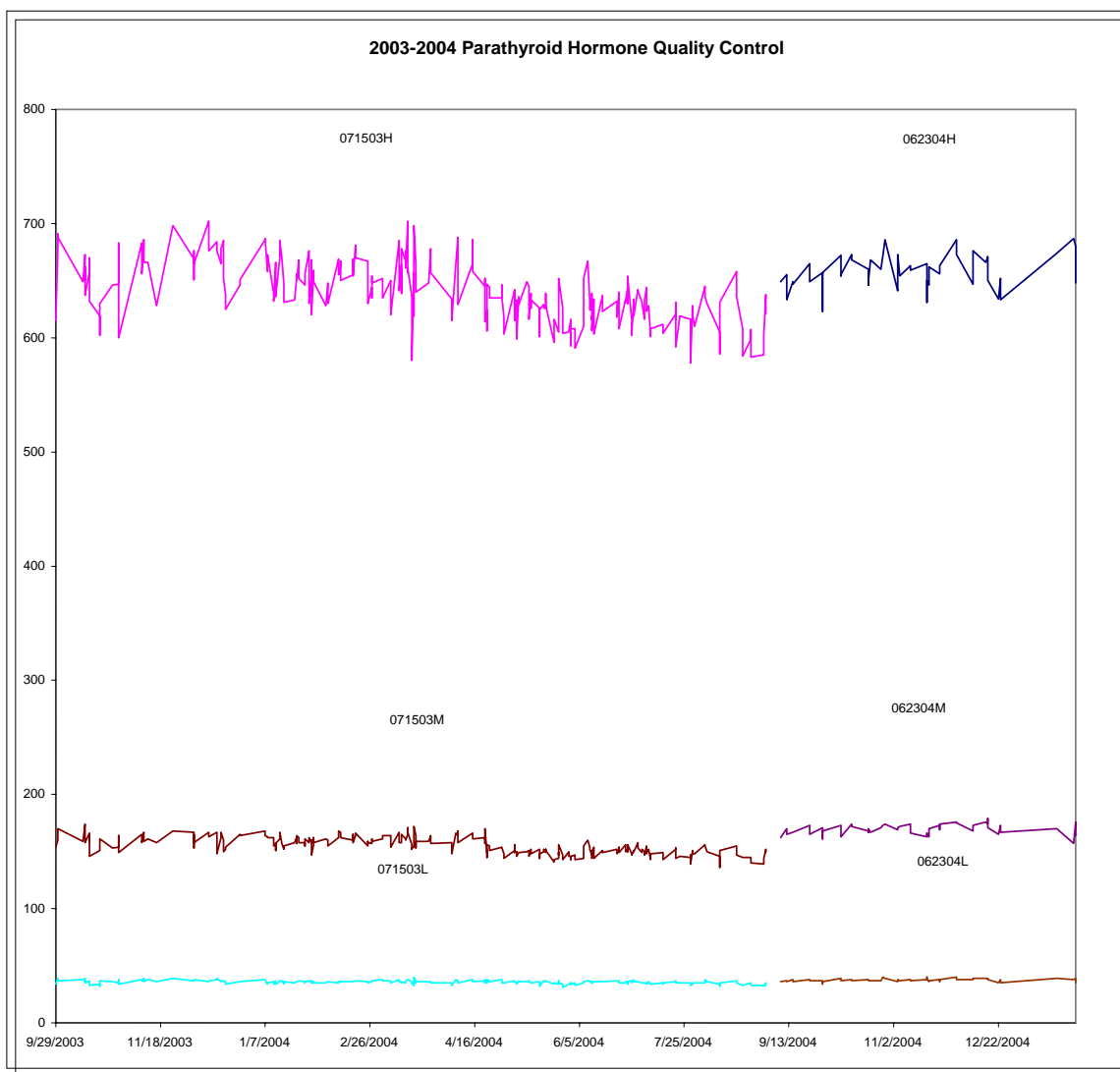
Standard record keeping should be used for tracking specimens. The primary results include daily test results as well as stored quality control results.

The original NHANES IV ship file is copied into a template Excel file and onto the hard drive of a PC computer. After the results are entered into the database and assay results transmitted electronically, files are stored for 6 months on a server that is backed up on a daily basis. After 6 months, the result files are transferred onto a CD along copies of original ship files and QC information.

The residual serum is stored at < -70°C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Parathyroid Hormone by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
071503	278	9/29/2003	9/2/2004	35.8	1.4	4.0
071503	278	9/29/2003	9/2/2004	154.9	7.5	4.8
071503	278	9/29/2003	9/2/2004	639.7	25.8	4.0
062304	51	9/9/2004	1/28/2005	37.5	1.3	3.6
062304	51	9/9/2004	1/28/2005	169.3	4.6	2.7
062304	51	9/9/2004	1/28/2005	657.7	14.9	2.3



REFERENCES

1. Roche PTH Assay Insert. Version 2002-02-2133091001 01 06.
2. Roche Elecsys 1010 Operator's Manual.

Other Sources:

Intra-Operative Parathyroid Hormone (Roche Elecsys 1010) Procedure. Revised May 2003.