

Laboratory Procedure Manual

Analyte: **Thyroxin (T4)**

Matrix: **Serum**

Method: **Hitachi 704**

Method No.:

Revised:

as performed by: *Coulston Foundation
Alamogordo, New Mexico*

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Important Information for Users

Coulston 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 2001–2002 data.

Two laboratories performed this testing during 2001–2002. In order to maintain confidentiality of the participants the quality control summary statistics and graphs were combined to mask the individual analysis dates from the two laboratories. Methods for both labs are included in this release. The method for Lab18 analyte is included in this file. The method for Lab40 is described in a separate file.

A tabular list of the released analytes follows:

| Lab Number | Analyte | SAS Label |
|-------------------|----------------|----------------------|
| lab18 | LBXT4 | Thyroxin (T4) µg/dL |
| lab18 | LBDT4SI | Thyroxin (T4) nmol/L |

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The intended use of this procedure is to provide a standardized method of processing NHANES T4 specimens on the Hitachi 704 chemistry analyzer.

Total T4 measurements are used alone, or in conjunction with other thyroid tests, in the diagnosis and treatment of thyroid disorders.

Elevated levels of T4 occur in Graves' disease, subacute thyroiditis, toxic nodule or secondary hyperthyroidism. Decreased levels occur in primary hypothyroid diseases such as Hashimoto's thyroiditis and neonatal hypothyroidism due to defects at the hypothalamic-pituitary level.

2. SAFETY PRECAUTIONS

- a. Consider all specimens for analysis as potentially positive for infectious agents including HIV and Hepatitis B virus.
- b. Observe universal precautions **AT ALL TIMES** while handling blood products.
- c. Wear appropriate personal protective equipment while working with blood products.
- d. Place contaminated disposable plastic, glass and paper in their appropriate biohazard container located at each workstation.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- a. The integrity of specimen and analytical data generated by this method is maintained by proofreading all transferred data from a printed copy of the output file and storing data in multiple computer systems. Data files containing the date, analytical run ID, specimen analytical results by specimen ID, and method code are stored in archive files in the Hitachi 917 main computer system in an ASCII format. Files are downloaded from the Hitachi 917 to the host computer (CompuAdd 386) via an RS232 port. The data are stored in two files: 1) the H_917.DBF, which contains all data received from the Hitachi 917 and includes all participant data and analytical results, and 2) the H_TABLE.DBF file, which contains the names of the tests, their respective Hitachi 917 test code numbers, and the date and time the samples were entered into the Hitachi 917 workstation. An output file, created by selecting fields from the NHANES files, is downloaded. An ASCII file of the data, created and copied to a 5¼" HD diskette, is sent to NCHS as an email attachment. The file is also copied onto another CompuAdd 386 in the laboratory administration area.
- b. Routine backup procedures include: 1) weekly backup of hard disks and 2) archival of data on a 3½" HD floppy diskette. Floppy diskettes containing sensitive data are stored in locked cabinets.
- c. Documentation for system maintenance is contained in hard copies of data recorded, as well as in files on the local tape drives used for archival of data.

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

- a. Use a nonhemolyzed specimen from a subject.
- b. Specimen type: serum or plasma with EDTA, heparin, citrate, or fluoride anticoagulants. Do not use oxalate.

Separate serum or plasma from cells within 1 hour of collection.

- c. The optimal amount of specimen is 1.0 mL of serum; the minimum is 0.5 mL of serum or plasma.
- d. Acceptable containers for collection include 10- or 15-mL red-top or serum-separator Vacutainer tubes. Store serum in 2.0-mL Nalge tubes.
- e. Specimens should be refrigerated if not used immediately. Specimens stored longer than 24 hours should be frozen at $\leq -20^{\circ}\text{C}$. Specimen stability has been demonstrated for 1 year at $\leq -20^{\circ}\text{C}$.
- f. The criteria for unacceptable specimens are low volume (<0.25 mL), hemolysis, improper labeling, and prolonged contact of serum or plasma with cells.
- g. Specimen handling conditions are outlined in the Coulston Foundation Laboratory's Collection Procedures and Specimen Requirements Manual located in all sections of the laboratory and available to clients upon request. Collection, transport, and special requirements are discussed. In general, serum specimens from NCHS collection sites are transported on dry ice and stored at $\leq -70^{\circ}\text{C}$ until analysis. Residual samples should be refrozen at $\leq -70^{\circ}\text{C}$.

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. Instrumentation: Hitachi 704 System

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Calibration is automatically performed by the analyzer.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. PRE-ANALYSIS PROCEDURES:

- 1) Perform all Hitachi 917 maintenance that is required for Daily Operation as required by the maintenance schedule in the Hitachi 917 Maintenance Log.
- 2) Prepare, if necessary and load T4 reagents on the Hitachi 917 in sufficient quantity to perform the necessary workload for the day.
- 3) Perform the morning Calibration and Quality Control verification of T4 per SOP CC-6.0.
- 4) Check INDIVIDUAL QC after the morning QC has printed off (per SOP CC-6.0) to verify if QC is acceptable to run NHANES specimens. Note: whenever possible, QC for each level (Precitrol Normal and Precitrol Abnormal) should be 1 Standard Deviation (SD) or better. Sometimes however, it is just not possible to keep QC within 1SD. If you have done **EVERYTHING** you can to improve the QC on T4 and it is within 2SD, proceed with processing NHANES T4 specimens. If QC is 2SD or GREATER, resolve this problem BEFORE processing NHANES and/or any other T4 specimens. If QC is acceptable and there are plenty of reagents on board, proceed to programming specimens.

b. PROGRAMMING SPECIMENS:

- 1) Touch the WORKPLACE button.
- 2) Touch the DATA REVIEW button.
- 3) Press the END key on the keyboard to go to the end of the Data File.
- 4) Verify the LAST sample number that was run. Your STARTING sample # will be the next one (i.e. if the last sample run is 1959, your first sample will be 1960).
- 5) On the Shipping Manifest Report, to the left of sample #1, write your starting sample # and starting position on the SAMPLE WHEEL (i.e. 1960, 1 for starting sample # 1960 and cup or position #1).
- 6) Continue numbering each successive specimen with the next sample # and the next position or cup # until you have assigned sample and position #'s to each specimen. **Note:** since T4 doesn't have an early reporting cutoff value, it is suggested to choose the specimens you are going to run as 2.5% Repeat specimens and program them too. **I would suggest choosing a specimen near the top of the manifest that way you have time to move it to its rerun spot after it has been sampled.**
- 7) Touch the Test Selection button on the screen to return to the sample ordering screen.
- 8) The Sample # box should already be highlighted (if not, touch that box to highlight it). Type your first sample # (i.e. 1960 from above) and press Enter on the keyboard.
- 9) The highlight will automatically move to the Disk # block. Type the number zero in this block and press Enter on the keyboard.
- 10) The highlight will automatically move to the Sample Position block. Type the number 1 in this block because you want to start at position or cup #1 on the sample wheel. Press Enter on the keyboard.
- 11) The highlight will automatically move to the Patient ID block. Type in the Sample/Vessel ID # of the 1st NHANES specimen on the Shipping Manifest Report and press Enter on the keyboard.
- 12) The highlight will automatically move to the test ordering section. Touch the T4 button. This action will highlight the T4 test. Note: do **NOT** press Enter on the keyboard or on the Test Selection Page **YET!**
- 13) Next you will order this test for all your remaining specimens by using the "REPEAT" button. Press the REPEAT button located at the bottom of the screen.
- 14) A repeat box opens up. In the Repeat Sample No. box, enter the very LAST Sample # of **ALL** the specimens you are going to process to **INCLUDE** your 2.5% Repeat specimens. The first thing to note is that for every **100** specimens processed, 2.5% of these specimens **MUST** be rerun. So for every 100 specimens processed, rerun **3** NHANES T4 specimens. Note: this VERY LAST SAMPLE # will be the very last sample on the last page of all your Shipping Manifest Reports plus your 2.5% Repeat specimens (i.e. if your last sample # is 2000, then type in Sample # 2000 and press Enter on the keyboard).
- 15) Press Enter on the keyboard again or press Enter in the Repeat box. The screen will say "In Process Please Wait".
- 16) When it is done, touch the Data Review button.
- 17) Press the End key on the keyboard to go to the very last sample that was ordered. Verify that the last sample ID # and sample position/cup position on the very last sample on the shipping manifest report match. If not, find the error and fix it.
- 18) You will need to enter the Sample/Vessel ID #'s for all your specimens with the exception of sample #1. To do this, touch the Test Selection button near the top left side of the screen.
- 19) In the Sample ID box, type in the sample # for sample #2 (i.e. if sample #1 was 1960, you will type in 1961) and press Enter on the keyboard. The test (T4) ordered for Sample #2 will appear but the Sample ID box will be blank.
- 20) Touch the Sample ID box to highlight it.

- 21) Type in the Sample/Vessel ID # for sample #2 and press Enter on the keyboard **TWICE**. This action will enter the Sample/Vessel ID # that you just typed and it will automatically move forward to Sample #3.
- 22) Touch the Sample ID box (Note: this box **MUST** be touched **EACH TIME** prior to typing a new Sample/Vessel ID #) and type in the Sample/Vessel ID # for sample #3 and Press Enter **TWICE**. Continue this process until the entire NHANES specimen Sample/Vessel ID #'s have been entered **including** the 2.5% Repeat specimens.
- 23) When you have finished programming your last sample, you need to print a REQUISITION LIST. Make sure that plain; single ply paper is in the printer. Touch the Print button at the bottom of the screen. The print page opens up.
- 24) touch the line that says "Requisition List" to highlight it.
- 25) Touch the Select button just below and slightly to the right. A requisition list box opens up.
- 26) In the First Sample No. box, type in your 1st sample # (i.e. 1960) and press Enter on the keyboard.
- 27) In the Last Sample No. box, type in your last sample # (i.e. 2000) and press Enter on the keyboard.
- 28) Press the Print button just below and your requisition list will be printed.
- 29) remove the list from the printer by pressing the Tear Off button on the top of the printer. This action will move the paper up to where you can tear it off at the perforation between pages.
- 30) Once you have removed the requisition list, press the Load button on top of the printer. This action will reset the paper to its normal position for printing specimen reports.
- 31) We want to save the printer ribbon as much as possible. So turn the printer **OFF** until after **ALL** of the T4 specimens are complete.

b. ANALYSIS PROCEDURE

- 1) Get the NHANES TSH/T4 specimen box from the refrigerator and place it on the counter.
- 2) Place the requisition list next to the specimen box and remove the lid from the box.
- 3) Remove specimen #1 from the box and compare the Sample/Vessel ID # on the vessel to the Sample ID # on the requisition list that you have next to the specimen box. If the requisition list doesn't match the vessel, make sure the Sample/Vessel ID # matches the Shipping Manifest Report. If it does, go to the 917 and correct the ID #. If the sample/vessel ID # doesn't match the Shipping Manifest Report, this should have been handled in SOP-11.0 when the specimens were received.
- 4) To correct a mistyped Sample/Vessel ID # on the 917, perform the following:
 - a. Touch the Work Place button if not already there:
 - b. Touch the Test Selection button if not already there.
 - c. In the Sample No. box, type the sample No. from the requisition list (it is the far left number on the same line the ID # is on, i.e. N01960, but do NOT type the N) and press Enter on the keyboard. That specimen will now be recalled on the screen.
 - d. Touch the Patient ID box, type in the correct Sample/Vessel ID # and press Enter on the keyboard **TWICE**. You have now corrected the ID #.
- 5) Mix/invert each specimen 5 to 10 times and then remove the cap.
- 6) Remove **ANY and ALL bubbles**, if present, from the specimen.
- 7) Inspect the serum for conditions that might affect test results (i.e. lipemia, hemolysis, icterus, etc). If the serum is NOT normal in appearance, utilizing the Specimen Comment Code list (provided by NHANES) that is hanging above the chemistry counter, record the code that best describes the condition and/or conditions of the serum to the right of that specimen on the Shipping Manifest Report.

- 8) After inspection, place the vial cap in the bottom of the Sample tube on the Sample Wheel in the proper position (i.e. sample #1 in tube #1) with the threaded end of the cap pointing down. Then place the sample vial on top of the cap.
- 9) Load **ALL** of the T4 specimens **PRIOR** to starting the analysis.
- 10) Once you have **ALL** of the T4 specimens loaded on, press the small START button located at the bottom left corner of the screen.
- 11) Then press the LARGE START button that appears on the next screen to start the analyzer. Note: sometimes the analyzer doesn't start, just press the small START button and the LARGE START button again and it should start this time.
- 12) Once the analyzer starts, touch the SAMPLE TRACK button at the bottom of the screen to switch to the sample monitoring/tracking screen. **Note: in order to leave this screen, you must touch the CLOSE button located on the right lower side of this screen.** As each specimen is scheduled for processing, the corresponding sample # indicator on the displayed wheel will turn gray. As each specimen is completed, the indicator will turn from gray to green to indicate this specimen is complete.
- 13) If the indicator does turn green, remove the specimen, recap it and place the specimen back into the specimen box it came in. If the indicator did **NOT** turn green, this indicates the specimen is **NOT** complete and will be scheduled for a rerun. The analyzer will automatically accomplish this. Leave the specimen on board until the indicator turns green.
- 14) Remove all complete specimens to keep the specimens as fresh as possible until all of the specimens have been completed and removed.

c. EDITING RERUN SPECIMENS

- 1) On occasion, specimens get rerun by the analyzer due to either being outside linearity or because a reagent was used up and the analyzer switched to a new reagent. When this occurs, that sample needs to be edited after the rerun is complete.
- 2) If you are still on the Sample Tracking screen, press the **CLOSE** button located on the lower right side of the screen. This action will close the Sample Tracking screen.
- 3) Touch the Work Place button.
- 4) Touch the Data Review button.
- 5) Press the End key on the keyboard to go to the end of the data file.
- 6) Using the up or down arrow keys on the keyboard, scroll to the specimen that needs editing.
- 7) Touch the T4 result in the Test Result section located on the right side of the screen to activate this section. If it was rerun, there will be a result displayed in the Rerun column.
- 8) Touch the Page button located on the lower right side of the screen until page 2 appears.
- 9) Touch the EDIT button. This action opens up an EDIT DATA screen.
- 10) In the EDIT DATA box, there will be results displayed in the 1st line, and results displayed in the Rerun line (if you had the highlight **ON** the test to be edited). Touch the button entitled "Replace" located below this box.
- 11) The analyzer will ask "Are You Sure?" Touch the Yes button to put the rerun value in the 1st line. This action will replace the original value with the rerun value. Note: the rerun value will now be displayed in both the 1st line and the Rerun line.
- 12) Since this is an NHANES run and due to the DATA Base that is used to process and transmit this information to NHANES, there **CANNOT** be a value greater than 0 (ZERO) in the rerun line. If you forget to change the rerun line to zero, when the Data is processed for transmittal to NHANES, the 1st value **AND** the Rerun value will be added together therefore giving an erroneous result. To prevent this, touch the Rerun line to highlight it.
- 13) Type the number zero in this box and press Enter on the keyboard. This action will store the zero value in the Rerun line.
- 14) Press the Close button on the screen. This returns you to the Data Review screen. Check to make sure the results are changed (i.e. the rerun value is now in the "Result" column and the zero value

is in the “Rerun” column. After you have finished editing any specimens that required editing, it is time to print **ALL** of the results.

- i. **Turn the printer back ON.**
- ii. Press the End key on the keyboard to go to the end of the Data file.
- iii. Using the up or down arrow keys, scroll up to the very 1st NHANES specimen you ran (i.e. 1960 as described earlier) making sure the highlight is on that specimen.
- iv. If only **ONE** container of specimens were processed, touch the “R” (for Range) button and then use the **DOWN** arrow key to scroll down to the **LAST** 2.5% rerun sample.
- v. Touch the very **LAST** sample that you highlighted (the **LAST** 2.5% Repeat sample). This action will cause all the samples to turn green. This indicates they are now selected.
- vi. If you processed more than one container of specimens, touch the “M” (for Mark) button and then touch **ONLY** the specimens that you ran from that container to include any 2.5% repeat specimens that were rerun from that container.
- vii. Touch the **PRINT** button located at the bottom of the screen.
- viii. Touch the **Select** button on the next screen because “Measurement Data” is already chosen by DEFAULT.
- ix. Touch the **MONITOR** button on the next screen that appears if it isn’t already selected.
- x. Touch the “**PRINT**” button to print the entire T4 run in **MONITOR** format because that is all that is required for T4 specimens.

d. POST-ANALYSIS PROCEDURES

- 1) After the analysis is complete, all reports have been printed, all specimens have been recapped and placed back in the refrigerator, it is time to review the reports for accuracy and correct information.
- 2) Remove the reports from the printer by pressing the Tear Off button on top of the printer. This action will move the paper up to where you can tear it off at the perforation between pages.
- 3) Once you have removed the reports, press the Load button on top of the printer. This action will reset the paper to its normal position for printing specimen reports.
- 4) Review **EACH** result and verify and/or perform the following:
 - i. Sample/Vessel ID # is correct (this should have already been done when comparing the ID #'s to the requisition list).
 - ii. Place a circle around the “**Ser**” on the line that says specimen type: Ser/PI for **EACH** T4 specimen.
 - iii. Write your initials and the date to the right of **EACH** T4 result.
 - iv. Check each T4 result to ensure it is within linearity of the Hitachi 917.

9. REPORTABLE RANGE OF RESULTS

The analytical range for Total T4 is to 0.5 µg/dL.

10. QUALITY CONTROL (QC) PROCEDURES

The method described in this protocol is in compliance with Clinical Laboratory Improvement Act of 1988 (CLIA) and the College of American Pathologists (CAP) guidelines. The Hitachi analyzer has proven to be accurate, precise, and reliable. Precision studies were generated from the use of a long-term lot number of quality control material.

Two types of quality control material are used to establish the Coulston Foundation quality control program: (1) a normal and abnormal level of quality control material assayed by the RD, and (2) the CAP Proficiency

Survey. All levels of this parameter are assessed by taking these samples through the complete analytical process. The data from these materials are used to estimate methodological imprecision, shifts, and trends.

The assayed quality control materials are used to form a normal and an abnormal level of each analyte for the Hitachi 704, manufactured by RD. These materials are lyophilized preparations of pooled human serum with added chemicals, human and animal tissue extract and preservatives. The controls are assayed by the RD which establishes concentrations for each lot and determines the mean and ± 2 SD ranges for each parameter. One set of assayed controls is analyzed at the beginning of the analyzer run period to verify calibration and as an accuracy check. If the inventory of these materials becomes low, another lot should be ordered in time to analyze it concurrently with the lot currently in use so that a bridge may be formed between the materials.

After the standards and the assayed quality control materials are analyzed, the 30-day Levey-Jennings control chart (which is stored in the Hitachi 917 computer system) is consulted to determine if the system is "in control." The system is out of control if any of the following events occur for any one of the parameters:

- a. Test the control data using the 2 SD rule. Accept the run when all controls are within 2SD of the mean. Report participants' results. When at least one control observation is more than 2SD from the mean, hold the participants' results and inspect the control data further, using additional Westgard control rules.
- b. Inspect control data within the run.
 - Reject the run when one observation is more than 3SD from the mean. Do not report participants' results.
 - Reject the run when two control observations are more than 2 SD on the same side of the mean. Do not report participants' results.
 - Reject the run when the range of one control observation is more than 2SD above the mean and that of another is more than 2SD below the mean. Do not report participants' results.
- c. Inspect control data across runs.
 - Reject the run when two consecutive control results are more than 2SD on the same side of the mean. Do not report participants' results.
 - Reject the run when four consecutive control observations fall more than 1SD on the same side of the mean. Do not report participants' results.
 - Reject the run when three consecutive control observations fall more than 1SD on the same side of the mean. Do not report participants' results.
 - Reject the run when 10 consecutive observations fall on the same side of the mean. Do not report participants' results.
 - Reject the run when 9 consecutive observations of the same control material fall on the same side of the mean. Do not report participants' results.
- d. Accept the run when none of the rules indicate a lack of statistical control. Report participants' results.

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

If one or more quality control samples fall outside the ± 2 SD range or a within-run control sample shifts 2 SD or more from its previous value, then take the following steps:

- a. Determine the type of errors occurring (random, systematic, or both) on the basis of the control rules being violated.
- b. Refer to the RD troubleshooting guide to determine the elements of the method or the components of the instrument that can cause the type of error observed.

- c. Correct the problem, then reanalyze the participants' samples and control samples, testing for statistical control by the same procedure.
- d. Consult the chemistry technical supervisor for any decision to report data when there is a lack of statistical control. The chemistry technical supervisor may make a decision to report data when there is a lack of statistical control in the following situations:
 - (1) The control problem can be due to the control materials themselves.
 - (2) The control problem has resulted from an isolated event that would not have affected the rest of the run.
 - (3) The control problem occurs in a concentration range that is different from the concentration range of the participants' samples. The method is in-control in the range of the participants' samples.
 - (4) The size of the analytical error is judged to be small relative to the medical usefulness requirements.
- e. PERFORMING QC AFTER NHANES PROCESSING
 - (1) **IMMEDIATELY** after you have finished processing ALL NHANES T4 specimens to include any reruns **AND** 2.5% Repeat specimens, QC **MUST** be performed.
 - (2) Perform Post NHANES Quality Control verification of T4 per SOP CC-6.0 remembering to put on **FRESH** QC material before you start the QC run.
 - (3) Check INDIVIDUAL QC after the QC has printed off (per SOP CC-6.0) to verify if QC is acceptable. Note: whenever possible, QC for each test should be 1 Standard Deviation (SD) or better. Sometimes however, it is just not possible to keep QC within 1SD. If QC is within 2SD, the NHANES run can be accepted. If QC is 2SD or GREATER, try rerunning QC on T4. If you cannot resolve this problem by rerunning QC or changing the reagent, you may need to recalibrate T4. If you do end up recalibrating, T4 will have to be **RERUN** on **EVERY** NHANES T4 specimen. If that happens, be sure to rerun QC on T4 again
 - (4) **AFTER** you rerun all the specimens.

After **T4** QC is acceptable; a QC INDIVIDUAL LIST needs to be printed. Perform the following:

 - i. Touch the QC button located at the top of the screen.
 - ii. Touch the Individual QC button.
 - iii. Touch the screen anywhere inside the large box to activate the screen.
 - iv. Press the **END** key on the keyboard to move to the end of the QC file.
 - v. Touch either the T4 P-N or the T4 P-A line to highlight it.
 - vi. Touch the **M** (for Mark) button located to the right of the QC file.
 - vii. Touch **BOTH** the T4 P-N **AND** the T4 P-A lines to turn them green. This action selects both QC levels.
 - viii. Touch the Print button located at the bottom of the screen.
 - ix. Touch the line in the new box that appears that says "Individual QC List" to highlight it.
 - x. Touch the Select button in this box.
 - xi. A new box opens that is entitled Individual QC list; press the Print button in this box. All of the T4 QC for the day will be printed.
 - xii. Remove this printout from the printer as described earlier and place it in the NHANES T4 folder.
 - xiii. Place the NHANES T4 folder and floppy disk on the Lab Managers desk for review.

12. 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. Measurement of Total T4 gives a reliable reflection of clinical thyroid status in the absence of binding abnormalities. Changes in binding proteins can occur which affect the level of Total T4 but leave the level of unbound hormone unchanged.
- e. Thyroid status should not depend on results from a single Total T4 test. Complete thyroid status evaluation should include additional thyroid function test and should be interpreted in light of the total clinical picture.

13. REFERENCE RANGES (NORMAL VALUES)

The reference range is 5.4–12.8.

14. CRITICAL CALL RESULTS (PANIC VALUES)

There are no critical call back values.

15. 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 on the IMX.

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.

16. 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.

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

Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as a comma-delimited file, either through electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.

Internet FTP transfers of files or dial up modem transfer options are available.

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

Microsoft Excel spreadsheets are used to keep records and track specimens analyzed. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

The Project Supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Thyroxine by Lot

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|-------|----|------------|------------|--------|--------------------|--------------------------|
| 15111 | 32 | 1/30/2001 | 2/14/2002 | 3.646 | 0.241 | 6.6 |
| 15112 | 32 | 1/30/2001 | 2/14/2002 | 17.085 | 0.427 | 2.5 |
| IMM1 | 41 | 7/23/2002 | 12/24/2002 | 3.64 | 0.28 | 7.7 |
| IMM2 | 41 | 7/23/2002 | 12/24/2002 | 6.68 | 0.37 | 5.5 |
| IMM3 | 41 | 7/23/2002 | 12/24/2002 | 15.39 | 0.61 | 4.0 |

2001-2002 Thyroxine Quality Control

