

Laboratory Procedure Manual

Analyte: Soluble Transferrin Receptor

Matrix: Serum

Method: Roche Tina-quant®

Roche Diagnostics Hitachi 912® system

Method No:

Revised:

as performed by:

Inorganic Toxicology and Nutrition Branch Division of Laboratory Sciences National Center for Environmental Health

contact:

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Important Information for Users

CDC 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.

Soluble Transferrin Receptor in Serum	
NHANES 2005-2006	

Procedure Change Log

Procedure: <u>Transferrin Receptor – Roche Tina-quant</u>®_ DLS Method Code: _____

Date	Changes Made	Ву	Reviewed By (Initials)	Date Reviewed
July 2005	P.2-4 - Changed write-up for instrument import of data into Access (started Jan 2005) to more clearly explain steps involved.	Irene Williams		
July 2005	P.7 - Changed sentence about frequency of inhouse PT checks from 3 to 2x per year	Christine Pfeiffer		
08/17/07	P.9 (reportable range) and P.10 (critical call results) – Changed upper bound for repeats from >5 to >5.5 mg/L.	Christine Pfeiffer		
08/17/07	P.15 – Corrected expected values according to Roche package insert (2.2-5.0 mg/L for men, 1.9-4.4 mg/L for women). Previous values were from an older insert.	Christine Pfeiffer		

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	
TFR_D	LBXTFR	Transferrin receptor	

1. Summary of Test Principle and Clinical Relevance [1-8]

The method principle for measurement of soluble transferrin receptor (sTfR) is immunoturbidimetry using Roche kits on the Hitachi 912 clinical analyzer. Latex bound anti-sTfR antibodies react with the antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically.

The transferrin receptor is an integral membrane glycoprotein having a molecular weight of 190 kilodaltons (kD). It consists of two identical subunits linked by disulfide bridges. Each of the monomers has an 85 kD C-terminal component which can bind an iron-laden transferrin molecule. Proteolysis leads to the soluble form of the transferrin receptor. In plasma, sTfR is present in the form of a complex with transferrin having a molecular weight of approximately 320 kD. The serum concentration of sTfR is directly proportional to the concentration of the receptor on the membrane.

The uptake of iron by the body's cells is controlled by expression of the transferrin receptor (TfR). If the intracellular iron stores are exhausted - corresponding to a ferritin concentration of less than 12 ug/L - more TfR is expressed. The affinity of the transferrin receptor to transferrin depends on the latter's loading state. As 80-95% of the transferrin receptor molecules are localized on erythropoietic cells, the TfR concentration (and hence also the sTfR concentration) reflects the iron requirement of the cells. When iron deficiency exists, the sTfR concentration in serum rises even before the hemoglobin concentration is significantly depressed. The sTfR concentration reflects the iron storage status. A precise assessment of the iron status can be obtained by determining the sTfR index (=sTfR concentration/log ferritin concentration).

As - in contrast to ferritin - the concentration of sTfR is not affected by acute phase reactions, such as acute liver function disorders or malignant tumors, it is possible to differentiate between anemia of chronic disease (ACD) and iron deficiency anemia (IDA). Elevated sTfR values are also found in polycythemia, hemolytic anemia, thalassemia, hereditary spherocytosis and vitamin B12 deficiency. Elevated sTfR concentrations occur during pregnancy when there is a deficiency of functional iron. Therapy with rhEPO can be monitored via the sTfR concentration.

2. Safety Precautions

Consider all plasma 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, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place disposable plastic, glass, and paper (pipet tips, autosampler vials, gloves, etc.) that contact plasma in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution 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.

If needed, MSDS for other chemicals can be viewed at http://www.ilpi.com/msds/index.html or at http://intranet.cdc.gov/ohs.

3. Computerization; Data System Management

A. Calculation of sTfR values are accomplished with the software on the Hitachi 912 instrument. Generated data is copied onto a floppy disk and archived to C: as an ASCII file. Transmission of data from A: to the Microsoft Access Network Database is described below:

Step 1 – Analyst – Import data file into ACCESS:

- Double click the ACCESS icon on desktop, password entry required
- [Add Sample Results to Database] (under Batch & X-Batch)
- [Import Instrument Data File] Enter information (instrument, assay, date, time, analyst, study)
- [Import] In "select data file" window, choose A: and import file number assigned for the date/run number. Check that sample ID's are recognized
- [Transfer]

Step 2 – Analyst – Review run in ACCESS:

- [Run Review] (under Batch & X-Batch) Select assay
- [Show runs] Cursor to desired run, enter sample set name and comments
- [QC Results] Review QC results for transmission errors and whether they pass the 2S limits
- [Print Report] [Back]
- [Sample Results] Review patient results to assure proper information transmission, enter appropriate comment codes on flagged samples
- [Set Final] results that are ready to be reported
- [Set Reviewed]
- [Print Report] [Back]

Step 3 – Analyst – Send email and run folder to QA Officer:

An e-mail is sent to the QA Officer including the following run information: Analysis date, Instrument, Study, Groups, File name, Batch ID, Run #, and QC Status. Noteworthy comments are included in the email. All printouts including raw data are submitted in a run folder to the QA Officer who reviews the Bench QC data via the ACCESS database as described below.

Step 4 – QA Officer – Review Bench QC via ACCESS:

- Double click the ACCESS icon on desktop, password entry required
- [Export QC to SAS] (under Batch & X-Batch) Select Assay, Date range and Controls
- [Make QC Data Infile] Save file to I:, appropriate subfolder for archival
- [Run SAS] SAS will automatically open, [go], review each generated plot, print QC cover page and standard deviation plot, [Back]

- [Run Review] (under Batch & X-Batch) Select assay
- [Show runs]
- [Sample Results]
- [Set Batch QC] accept or reject
- [Set Reviewed]
- Forward email from Analyst to Second QA reviewer (for Blind QC review) specifying Bench QC status of the run.

Step 5 – Second QA Reviewer – Review Blind QC and other parameters in ACCESS:

- Double click the ACCESS icon on desktop, password entry required
- [Run Review] (under Batch & X-Batch) Select assay, then desired run
- [Blind QC Results] Review whether Blind QC results pass the 2S limits
- [Print Report] [Back]
- Check other parameters if applicable (i.e., background, calibration curve, repeat values, replicates, signal intensity)
- [Set RQC] accept or reject
- Verify that appropriate comment codes have been applied and that final values have been set correctly
- [Set Reviewed]
- Forward email from QA Officer to Supervisor specifying Blind QC status of the run and other relevant comments.

<u>Step 6 – Supervisor – Approval and Export of Results via ACCESS:</u>

- Double click the ACCESS icon on desktop, password entry required
- [Run Review] (under Batch & X-Batch) Select assay, then desired run
- Perform final review of Bench and Blind QC status, comment codes, repeat results
- [Set Ready] Final results will be set ready to be exported
- [Set Reviewed]
- [Export/Report Results] (under Study Functions) Select study, select analytes/panel, use selected panel
- [Generate Excel Spreadsheet] Review file on I:\To be transmitted
- [Generate Export Text File and Set Results Exported] Review file on I:\To be transmitted
- FTP file to Westat
- Send Westat an email that file was transmitted
- After min. of 1 day, move transmittal file from I:\To be transmitted to I:\Transmitted Data\Appropriate Year Folder.

For NHANES, data is transmitted electronically several times weekly to Westat's ISIS computer system and transferred from there to NCHS. Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file to the Westat ISIS computer, and are eventually

- forwarded to NCHS. Westat also prepares the abnormal report notifications for the NCHS Survey Physician.
- B. Files stored on the network or CDC mainframe are automatically backed up nightly by DLS LAN support staff and CDC Data Center staff, respectively. Backup of the daily data containing all raw data files and result files for each run are the responsibility of the analyst. Typically these files are backed up once a week onto a floppy disk or a CD-ROM using a CD writer.
- C. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

- A. Use serum or plasma collected by standard venipuncture technique. Lithium and sodium heparin plasma may be used.
- B. All samples may be stored for 3 days at 20-25 °C, 7 days at 2-8 °C or 4 weeks at -20 °C (if only one freeze/thaw).
- C. Centrifuge samples containing precipitate before performing the assay.
- D. A 500- μ L sample of serum/plasma is preferable to allow for repeat analyses; a volume of 150 μ L is required for analysis.
- E. The appropriate amount of serum/plasma is dispensed into a Nalge cryovial or other plastic screw-capped vial labeled with the participant's ID.
- F. Specimens collected in the field are frozen, and then shipped on dry ice by overnight mail. Frozen samples are stored at -70 °C. Samples are stable for at least 5 years if stored at ≤20 °C [7] and can withstand 2 freeze/thaw cycles [6].
- G. Specimens generally arrive frozen. Refrigerated samples may be used provided they are kept cold and brought promptly (within 2 hours) from the site of collection.
- H. Specimens that have been through more than two freeze-thaw cycles, have been refrigerated for more than one week, undergone hemolysis, or contain particulate matter may give inaccurate results.
- I. Specimen handling conditions are outlined in the Policies and Procedures Manual of DLS (copies are available in the Nutritional Laboratory and the electronic copy of this file is located at Q:/ITN/Nutrition Laboratory/CLIA). The protocol discusses collection and transport of specimens and the special equipment required. In general, plasma should be transported and stored at no more than -20 °C. Samples thawed and refrozen less than twice are not compromised. If there is more than one analyte of interest in the specimen and it needs to be divided, the appropriate amount of blood or plasma should be transferred into a sterile Nalge cryovial labelled with the participant's ID.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagent Preparation

The assay is performed exactly as outlined by the manufacturer. All reagents are supplied by Roche Diagnostics in liquid form ready to be used. If the entire reagent kit is not used in one run, store the kit at 2-8 °C on the analyzer until the expiration date of the reagent is reached or the reagent has been on the analyzer for 90 days, whichever comes first.

B. Standards Preparation

0.9% NaCl is used as the zero standard and 5 other standards are supplied by Roche Diagnostics in liquid form with concentrations varying between calibrator kit lot numbers. Store the standards at 2-8 °C until the expiration date of the kit.

C. Preparation of Quality Control Materials

1. Roche QC pools:

Commercially prepared quality control material is purchased from Roche Diagnostics in two levels (level I at 2.2 mg/L and level II at 7.3 mg/L). Controls are in liquid form and ready to use. Unopened controls are stable at 2-8 °C until expiration date. Once opened, controls are stable for 7 days at 2-8 °C. Controls should be allowed to come to room temperature before analyzing. Control values are lot specific and values of each new lot should be entered into the Hitachi 912[®] before analyzing a new lot number of control.

2. CDC QC pools:

Low and high serum based QC pools are prepared and characterized in-house. Means plus range limits for all pools are established by analyzing duplicates for at least 20 consecutive runs.

The low QC pool is prepared by selecting and pooling serum that contains low levels of sTfR (~2-3 mg/L) to represent a sample with normal sTfR concentration. The high QC pool is prepared by selecting and pooling serum that contains elevated levels of sTfR (~5-7 mg/L) to represent a sample with iron deficiency.

D. Other Materials

The following materials are all provided by the manufacturer (Roche Diagnostics):

- 1. Sample trays
- 2. Sample cups (standard and micro)
- 3. Probe / electrode

- 4. Cells
- 5. Wash solutions (probe wash, basic wash, cell wash)
- 6. Hitergent
- 7. Lamp
- 8. Diluent
- 9. System Cleaning Solution
- 10. Reagent Adapters
- 11. Calibrators (2-6) and Controls (I and II)

E. Instrumentation

- 1. Roche Diagnostics Hitachi 912[®] system (Roche Diagnostics, Indianapolis, IN)
- 2. Daigger Vortex Genie 2 (VWR, Suwanee, GA)
- 3. Multi-tube vortexer (VWR, Suwanee, GA)
- 4. Eppendorf micropipet (Brinkmann Instruments Co., Westbury, NY)

7. Calibration and Calibration Verification Procedures

The reagents are stable for 90 days on the analyzer or until the expiration date on the reagent bottle is reached, whichever comes first.

The Hitachi 912® system must be recalibrated if any of the following events occur:

- A new lot of reagent is started.
- One or more QCs are out of specification.
- Any dispense system component is replaced or any major maintenance is performed on the instrument to include replacing the lamp.
- Cells are replaced.
- A CALIB error occurs indicating a difference in current values from previously stored values of >20%. Check QC and if in control no recalibration is necessary.
- A STD?, SENS? Or CALERR flag. Error codes let you know what aspect of calibration failed. Errors should be investigated and corrected and calibration repeated.

Calibration is performed by placing calibrators 1-6 and controls in the pre-assigned calibration and control locations on the inner sample wheel compartment. The calibrators are loaded onto the wheel with the saline blank (diluent) in position S18. The remaining calibrators are loaded into positions S21-S25 (check calibration installation screen for definite calibrator locations). Instrument is programmed to do a start-up calibration; the calibrators are automatically analyzed, and the controls will be run before any patient samples are analyzed. **Patient samples may be run with the calibration.**

NIST or other reference materials are not available for sTfR assays.

There is also no external proficiency testing program available for sTfR. CDC is performing an in-house proficiency testing at least twice a year.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

- 1. Allow frozen serum/plasma (patient samples) to reach ambient temperature.
- 2. Perform the required maintenance of the Hitachi 912° system (monthly, weekly, daily).

B. Creating an Order List

- 1. Be sure to clear all previously programmed samples from the Work list menu screen.
- 2. In the "Work Place" menu, select the beginning sequence, disk and position numbers. Select appropriate sample type, sample cup and volume types. Select routine or STAT sample and then select test to be performed on the sample and enter or scan the patient barcoded ID number. Once you have completed programming each sample select "Accept/Next" button to program the next sample. The instrument will assign each sample a position number in sequence to the beginning number.

C. Preparing the Run

One run is defined as **one carousel** (max. 50 samples per carousel). Controls are run from the inner control wheel at the beginning of the first carousel (set 1) and the end of the first carousel (set 2). The controls, run at the end of the first carousel (set 2 for run 1), are also the set 1 controls for run 2.

Each run must contain two levels (low and high) of CDC sTfR QC pools at the beginning of the run before patient samples are run and at the end of the designate run.

When performing small runs or confirmation (repeat) runs, both levels of CDC sTfR QC pools must be run in duplicate.

- 1. Place empty micro sample cups onto 16 X 100 tubes in a test tube rack and pipette 150 μL of the serum samples into the sample cup. Be sure to load the samples from left to right.
- 2, For a calibration run: Pipette 150 μ L of calibrator into the sample cup, and place in designated calibrator positions on the inner wheel.
- 3, For runs other than calibration run: Pipette 150 μ L of both levels of control into each sample cup, and place in designated control position on the inner wheel. Pipette 150 μ L of patient sample into each sample cup and place on the instrument in the order of the work list starting with position 1 and ending with position 50 for the first run.

- 4. Ensure that no air bubbles are present in the sample cups. Break a wood applicator into pieces and use them to pop the bubbles.
- 5. Ensure that the amount of reagents, diluent, and wash solutions is adequate for the amount of samples to be run. You may place more than one bottle of reagent at a time on the analyzer; however, avoid using more than one lot number of reagent for a single run.

D. Initiating a Run

NOTE: Be sure to archive all previous test results before starting a new run and/or be sure to change the tray number.

Be sure that all sequence and tray numbers match.

- 1. For all runs, go to the start conditions screen, enter the number of the sequence you wish to start with (should correspond to your order/work list).
- 2. Select "Start Up Calibration" (this has to be done to get controls and QC to run from the inner wheel).
- 3. Once all necessary items are selected on this menu, press the "Start Button" on the screen.

E. End of Assay

Once results are complete, the instrument will automatically print the results. Review all results from the "Data Review" screen, then highlight all results and select *archive*. This will archive all results to the database for future retrieval.

NOTE: Be sure to check Data Review screen before starting a new run. Make sure that all results have been archived.

F. System Maintenance

The system maintenance consists of daily, weekly, and monthly maintenance.

- 1. Daily maintenance (Section 4-29 to 44 of the Hitachi 912® User's Guide Manual) should be performed at the start of each 8-hour shift, or more frequently, if necessary. It consists of performing a probe wash and clean, photometer check, cleaning and adjusting stirrers and probes and checking the printer paper supply along with checking reagent, wash and diluent volumes and reagent compartment temperatures.
- 2. Weekly maintenance (Section 4-45 to 47 of the Hitachi 912[®] User's Guide Manual) consists of performing cell wash, cell blank and cleaning cell rinse unit nozzles and probe and stirrer rinse baths.
- 3. Monthly maintenance (Section 4-48 to 63 of the Hitachi 912[®] User's Guide Manual) consists of replacing reaction cells, cleaning the bath and filter, cleaning sample and reagent disk compartments, cleaning ISE drain Port, cleaning refrigerator condenser filter and replacing ISE measuring cartridge.

G. Special Method Notes

1. The Hitachi 912[®] system should always be "put to sleep" when not in use.

2. Turn the system completely off <u>only</u> if you will be taking the instrument apart or when indicated by maintenance procedure or error code.

H. Calculations

All calculations are performed by the Hitachi 912[®] Software system using a machine-stored calibration curve.

9. Reportable Range of Results

Maximum reportable range is dependent on the concentration of the highest standard. For the purposes of CDC reporting, we will use a reportable range of 0.5-40 mg/L. Samples with values of >5.5 mg/L are automatically reanalyzed using routine (normal) sample volume.

No instrument flag will be generated on results that fall below the reportable lower range of the assay. In the event that a sample result falls between the lower reportable range and -99999 with no data flag, this result will be used in a calculated or compensated test regardless of where the qualitative limit is set.

10. Quality Control (QC) Procedures

A. Blind Quality Controls

Blind QC specimens are inserted prior to the arrival of the samples in the Inorganic Toxicology and Nutrition Branch. These specimens are prepared at two levels so as to emulate the patient samples; the labels used are identical to those used for patient samples. One blind QC specimen randomly selected for concentration is included at a randomly selected location in every 20 specimens analyzed.

B. Bench Quality Controls

Bench QC specimens are prepared from two serum pools, which represent low and high levels of sTfR in serum. These pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run.

The results from the pools are checked after each run. The system is declared "in control" if both QC results are within 2s limits and the run is accepted. If one of the two QC results is outside the 2s limits then apply rules below and reject if any condition is met - the run is then declared "out of control":

- 1_{3s} Any of the two QC results is outside the 3s limit
- 2_{2s} Both QC results in the run are outside the 2s limit (same side of mean)
- R_{4s} Sequential QC results (either within the run or across runs) are outside the 2s limit on the opposite sides of the mean
- 10_x Ten sequential QC results (across pools and across runs) are on the same side of the mean.

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC.

The initial limits are established by analyzing pool material in 20 consecutive runs and then reevaluated quarterly. When necessary, limits are updated to include more runs.

While a study is in progress, QC results are stored in the ACCESS database. For runs that are not imported into ACCESS (exception, research-type runs), QC results are stored electronically in the analyte-specific folder on Q:\ITN\Nutrition Lab\Data handling\QC results in Excel. A hardcopy of the QC results from each run is also kept by the analyst.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

Check to make sure that the hardware is functioning properly.

Recalibrate the instrument.

If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

Only one freeze/thaw cycle is recommended. Samples containing particulate matter should be centrifuged and the material removed before analysis. Results are not known to be affected by icteric, hemolyzed or lipemic specimens.

13. Reference Ranges (Normal Values)

sTfR reference ranges are assay-specific. The Roche Diagnostics kit specifies expected values of 2.2-5.0 mg/L for men and 1.9-4.4 mg/L for women.

14. Critical Call Results ("Panic Values")

The collaborating agency with access to patient identifiers or the responsible medical officer is notified by FAX by the supervisor of any sTfR result that is >5.5 mg/L, which possibly represents iron deficiency. Copies of FAXes sent concerning abnormal results are kept in a notebook by the supervisor for the duration of the study. For NHANES 1999+, since data are transmitted several times weekly to the Westat ISIS computer, Westat automatically notifies the NCHS survey physician.

15. Specimen Storage and Handling during Testing

Specimens are allowed to reach room temperature during preparation. The unused portion of the patient specimen is returned to the freezer.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

If the analytical system fails, we recommend that the specimens be stored at -20°C until the analytical system is restored to functionality.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

The collaborating agency with access to patient identifiers or the responsible medical officer is notified by FAX by the supervisor of any sTfR result that is >5.0 mg/L, which possibly represents iron deficiency. Copies of FAXes sent concerning abnormal results are kept in a notebook by the supervisor for the duration of the study.

Test results that are not abnormal 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 an ASCII text file, either through electronic mail or on a diskette.

For NHANES 1999+, all data are reported electronically several times weekly to the Westat ISIS computer and then are transferred to NCHS. For some smaller studies, hard copies of a data report are sent, as well as the results in electronic format.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

The Microsoft Access database is used to keep records and track specimens for NHANES 1999+. Records are kept in files on Q:\ITN\Nutrition Lab on the DLS LAN.

We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies may be discarded at the request of the principal investigator, or may be transferred to the CDC CASPIR facility for use by other investigators. Very little residual material will be available after NHANES analyses are completed, and these vials may be routinely autoclaved.

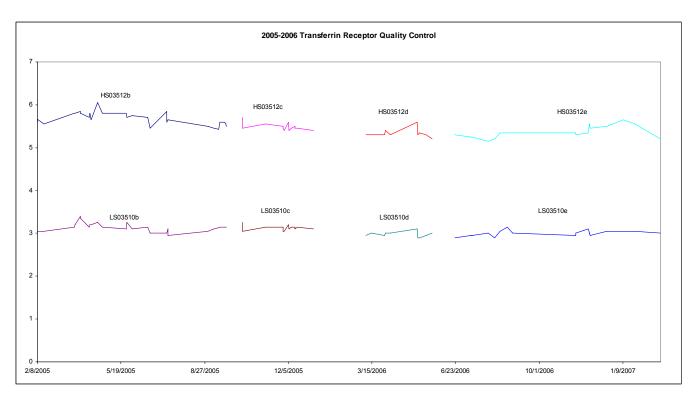
The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored in a freezer at -70°C. The specimen ID is read off of the vial by a barcode reader attached to the

computer used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the DIF file containing the electronic copy of the results is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping a notebook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies.

19. Summary Statistics and QC Graphs

Summary Statistics for Transferrin Receptor by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
LS03510b	28	2/8/2005	9/22/2005	3.13	0.10	3.3
HS03512b	28	2/8/2005	9/22/2005	5.67	0.15	2.6
LS03510c	13	10/11/2005	1/4/2006	3.13	0.06	1.9
HS03512c	13	10/11/2005	1/4/2006	5.48	0.09	1.6
LS03510d	10	3/8/2006	5/26/2006	2.98	0.06	2.0
HS03512d	10	3/8/2006	5/26/2006	5.34	0.11	2.0
LS03510e	17	6/22/2006	2/23/2007	3.00	0.07	2.2
HS03512e	17	6/22/2006	2/23/2007	5.36	0.14	2.6



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