



# Laboratory Procedure Manual

*Analyte:* **Ferritin**

*Matrix:* **Serum**

*Method:* **Roche E-170 Ferritin "ECLIA"**

*Method No:* 4046.01

*Revised:*

*as performed by:*

Nutritional Biomarkers Branch (NBB)  
Division of Laboratory Sciences  
National Center for Environmental Health

*contact:*

[James L. Pirkle, M.D., Ph.D.](#)  
[Director, Division of Laboratory Sciences](#)

## **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.

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**Public Release Data Set Information**

This document details the Lab Protocol for testing the items listed in the following table:

<b>Data File Name</b>	<b>Variable Name</b>	<b>SAS Label</b>
FERITIN_F	LBXFER	Ferritin (ng/mL)
	LBDFERSI	Ferritin (µg/L)

## **1. Summary of Test Principle and Clinical Relevance**

The method for measurement of Ferritin on the Roche Elecsys-170 is a sandwich principle with a total duration time of 18 minutes. The 1<sup>st</sup> incubation uses 10 uL of sample, a ferritin-specific antibody and a labeled ferritin-specific antibody to form a sandwich complex. The 2<sup>nd</sup> incubation occurs after the addition of microparticles that cause the complex to bind to the solid phase. 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. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve.

Ferritin has a molecular weight of 440 kD, depending on the iron content, and consists of a protein shell (apoferritin) that is composed of 24 subunits and an iron core containing an average of 2500 Fe<sup>3+</sup> ions (in liver and spleen ferritin) [1]. Ferritin tends to form oligomers, and when it is present in excess in the cells of the storage organs, there is a tendency to condense in the lysosomes to form semicrystalline hemosiderin. At least 20 isoferritins can be distinguished with the aid of isoelectric focusing [2]. This microheterogeneity is due to the differences in the contents of the acidic H and weakly basic L subunits. The basic isoferritins are responsible for the long-term iron storage function, and are found mainly in the liver, spleen, and bone marrow [1,3]. Acidic isoferritins are found mainly in the myocardium, placenta, and tumor tissue. They have a lower iron content, and presumably function as intermediaries for the transfer of iron in various syntheses [4-6].

Ferritin determinations are useful in evaluating iron metabolism and determinations at the beginning of therapy provide a measure of the body's iron reserves. A storage deficiency in the reticulo-endothelial system (RES) can be detected at a very early stage [7]. Clinically, a threshold value of 20 ng/mL has proved useful in the detection of prelatent iron deficiency and provides a reliable indication of exhaustion of the iron reserves available for hemoglobin synthesis. Latent iron deficiency is defined as a fall below the 12 ng/mL ferritin threshold. The two values are diagnostic even when the blood picture is still morphologically normal. A depressed ferritin level accompanied by hypochromic, microcytic anemia indicates manifest iron deficiency [1].

When the ferritin level is elevated and the possibility of a distribution disorder can be ruled out, this is a manifestation of iron overloading in the body. The ferritin threshold value used for this is 400 ng/mL. Elevated ferritin values are also encountered with the following tumors: acute leukemia, Hodgkin's disease and carcinoma of the lung, colon, liver, and prostate. Ferritin determinations have also proved to be of value in liver metastasis. Reasons for the elevated values could be cell necrosis, blocked erythropoiesis or increased synthesis in tumor tissue.

## **2. Safety Precautions**

Consider all plasma/serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats, 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/serum 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,

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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

Calculation of Serum Ferritin values are accomplished with the software on the Hitachi Mod PE instrument. A backup copy of the generated data is copied onto the zip drive as a binary file. The data is also copied onto a floppy disk and archived to [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_LABS\Instrument Results](#) as an ASCII file. Transmission of data from floppy disk A: to the Microsoft Access FrontEnds Database is described below:

#### Step 1 – Analyst – Import data file into FrontEnds:

- Double click the FrontEnds icon on desktop, password entry required
- [Add Sample Results to Database] (under Batch & X-Batch)
- [Import Instrument Data File] - Enter information (instrument, assay, 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 FrontEnds:

- [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 FrontEnds database as described below.

#### Step 4 – QA Officer – Review Bench QC via FrontEnds:

- Double click the FrontEnds 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]

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- Forward email from Analyst to Second QA reviewer (for Blind QC review) specifying Bench QC status of the run.

### Step 5 – Team leader – Review Blind QC and other parameters and set results ready in FrontEnds:

- Double click the FrontEnds icon on desktop.
- [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, that final values have been set correctly and that repeat results are as expected
- [Set Ready] – Final results will be set ready to be exported
- [Set Reviewed]
- Forward email from QA Officer to Supervisor specifying that results can be exported; include any relevant comments.

### Step 6 – Supervisor – Approvals and Export of Results via FrontEnds:

- Double click the FrontEnds icon on desktop, password entry required
- [Run Review] (under Batch & X-Batch) – Select assay, then desired run
- Perform a final review
- [Set Reviewed]
- [Export/Report Results] (under Study Functions) – Select study, select analytes/panel, use selected panel
- [Generate Pre-Export Text File] – Review file on [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_OC\QA\Data handling\To be transmitted](#)
- [Generate Export Text File and Set Results Exported] – [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_OC\QA\Data handling\To be transmitted](#)
- FTP file to Westat
- Send Westat an email that file was transmitted
- Move transmittal file from [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_OC\QA\Data handling\To be transmitted](#) to [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_OC\QA\Data handling\Transmitted\Appropriate Year Folder](#).

For NHANES, data is transmitted electronically 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.

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 regularly onto a floppy disk or a CD-ROM using a CD writer.

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. Li-, sodium heparin and K<sub>3</sub>-EDTA plasma may be used.
- b. The appropriate amount of serum/plasma is dispensed into a Nalge cryovial or other plastic screw-capped vial labeled with the participant's ID.
- c. 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 up to 2 weeks at 2-8°C [8] and 12 months at -20°C [9]. Multiple freeze/thaw cycles are generally not recommended, however samples can withstand up to 3 freeze/thaw cycles [8]. Refrigerated samples may be used provided they are kept cold and brought promptly (within 2 hours) from the site of collection.
- d. Centrifuge samples containing precipitate before performing the assay.
- e. A 500-µL sample of serum/plasma is preferable to allow for repeat analyses; an initial volume of 150 µL is required for the sample cup; 10 µL is used for analysis.
- f. Ensure patient samples, calibrators, and QC is at ambient temperature (20-25°C) before measurement.
- g. Because of possible evaporation effects, samples, calibrators, and QC on the analyzers should be measured within 2 hours.
- h. Specimens that 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 \\cdc\project\CCEHIP\_NCEH\_DLS\_NBB\_LABS\CLIA\DLS Policies and Procedures Manual). 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 labeled 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**

All reagents are supplied by Roche Diagnostics in a ready-for-use unit that cannot be separated. Every Elecsys Ferritin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot.

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Store the reagent kit **upright** in order to ensure complete availability of the microparticles. Bring the cooled reagents to approximately 20°C (45 minutes at room temp) and open the lids slightly before placing on the reagent disk of the analyzer. The reagent kit is stable after opening for 12 weeks at 2-8°C, on the analyzer for 6 weeks or until the expiration date, whichever comes first.

**b. Standards Preparation**

Elecsys Ferritin CalSet is supplied by Roche Diagnostics in liquid form. The predefined master curve is adapted to the analyzer by the use of Elecsys Ferritin CalSet. Store the standards at 2-8 °C until the expiration date of the kit.

**c. Preparation of Quality Control Materials**

1) Roche QC pools:

Elecsys PreciControl Anemia [10] can be used for quality control of the Elecsys Ferritin immunoassay on the E-170 analyzer. This is a lyophilized control serum based on human serum matrix in three concentration ranges. The lot specific values need to be entered into the Mod PE before analysis. Carefully dissolve the contents of one bottle of each level by adding exactly 2.0 mL of distilled water and allow standing closed for 30 minutes to reconstitute. Mix carefully, avoiding the formation of foam. Transfer aliquots of the reconstituted controls into empty snap-cap bottles (ControlSet Vials). Attach the supplied labels to these additional bottles. Aliquots intended for storage at -20°C should be frozen immediately and are stable for 1 month (freeze only once). Controls stored at 2-8°C are stable for 3 days. Ensure the controls are at ambient temperature before use.

2) CDC QC pools:

Low, Medium 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 Ferritin (~67-96 ng/mL) to represent a sample with normal Ferritin concentration.

The medium QC pool is prepared by selecting and pooling serum that contains mid- levels of Ferritin (~150-200 ng/mL). The high QC pool is prepared by selecting and pooling serum that contains high levels of Ferritin (~370-500 ng/mL).

**d. Other Materials**

The following materials are required but must be purchased separately from the manufacturer (Roche Diagnostics):

- 1) Sample racks
- 2) Sample cups (standard)
- 3) PC/CC Cups
- 4) Assay Tip/Assay Cup Combimagazine M
- 5) Wash and buffer solutions (ProCell M, CleanCell M, ProbeWash M, PreClean M)
- 6) Elecsys SysClean
- 7) Waste liners
- 8) Diluent Universal

**e. Instrumentation**

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- 1) MODULAR ANALYTICS E170<sup>®</sup> system (Roche Diagnostics, Indianapolis, IN)
- 2) Daigger Vortex Genie 2 (VWR, Suwanee, GA)
- 3) Eppendorf micropipet and tips (Brinkmann Instruments Co., Westbury, NY)

### 7. Calibration and Calibration Verification Procedures

The initial calibration should be performed using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Calibration is recommended every 28 days when using the same reagent lot or every 7 days if using the same reagent kit. The reagents are stable for 6 weeks on the analyzer, 12 weeks at 2-8°C or until the expiration date on the bottle of reagent is reached, whichever comes first.

Calibration can be manually programmed on the Calibration Status screen or calibration can be requested via the graphical icon representing the system in the status line. If the Calibration and QC Select button is yellow, click on it to display the Calibration and QC Select window. Touch the yellow button to order the “Recommended” Calibration and select the reagent bottles to be QC checked. (routine, backup or both). Load the required calibrators (Ferritin CalSet) in a calibration rack (black) and controls in their assigned (white) QC rack positions and place the racks on the loader. Press the Start (global button) and then press “Start” on the Start Conditions screen.

***NOTE: Verify that calibration and QC results are acceptable before measuring routine samples.***

The Elecsys 170<sup>®</sup> 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.
- A “NG” error indicates a failed calibration due to monotony of curve, minimum acceptable difference or system errors. Check for proper calibrators and order in rack or system problems. Recalibrate after corrections are made. If you find no other problems and calibration continues to fail, replace the reagent kit.
- Deviation of “dupl” error means the difference between the duplicate measurements is too large and the appropriate calibrator will be flagged. Check for bubbles in the calibrators or prime the E module. Repeat calibration.
- Factor – curve position check compared to the most recent lot calibration. (L-calib utilizes a calibration factor of 1) For all subsequent reagent pack calibrations (R-calib), a new calibration factor is calculated. If failures occur, replace the reagent pack.

CDC participates in the College of American Pathologists’ Proficiency Testing Program:

- “C” series for General Chemistry is 3 times per year
- “K” series for Ligand-Endocrinology is 3 times per year
- LN5 Ligand Cal V/L series is twice a year.

The **NIBSC Code 94/572 3<sup>rd</sup> International Standard for Ferritin, Recombinant** ([NIBSC 94-572 Serum ferritin recombinant.pdf](#)) is available for calibration verification.



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Recovery studies, including a published study, to assess traceability of the Elecsys Ferritin assay to more recent international standards (2nd IS 80/578 and 3rd IS 94/572) have been conducted, with results showing very good agreement [11].

### 8. Procedure Operating Instructions; Calculations; Interpretation of Results

#### a. Preliminaries

- 1) Allow frozen serum/plasma (patient samples), calibrators and QC to reach ambient temperature.
- 2) Perform the required maintenance on the E170<sup>®</sup> system (monthly, weekly, daily).
- 3) Make sure the analyzer and the test required is not masked.
- 4) If you will be running the same tests on all samples, go to the “Start” global button and set the default profile.
- 5) Be sure to clear all previously programmed samples from the Data Review screen after backing up the data.

#### b. Preparing the Run

*One run is defined as 100 samples or less. Controls are run at the beginning and the end of each run.*

*Each run must contain three levels (low, medium and high) of CDC Ferritin 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, all levels of CDC Ferritin QC pools must be run in duplicate.*

**NOTE: Be sure to backup all previous test results and clear the “data review” screen before starting a new run.**

- 1) Before running samples, ensure that no air bubbles are present in the sample cups. Break a wood applicator into pieces and use them to pop the bubbles if necessary.
- 2) For a calibration run: Open the barcoded calibrators and place them in an unassigned **black** calibrator rack. Nonbarcoded calibrators must be pipetted into sample cups and placed in their pre-assigned positions in **black** calibrator racks. When calibration is completed, the results will be printed.
- 3) To run QC: When running manufacturer QC's, open the barcoded QC's and place them in the **white** assigned rack and position. For nonbarcoded QC, pipette 150  $\mu$ L of QC into a sample cup and place atop the tube in the pre-assigned control position of the **white** QC racks. When the instrument is started, it will automatically run the correct tests on the QC and print the results.
- 4) To run samples. Place empty sample cups onto barcode labeled 13 X 75 tubes in **gray** sample racks and pipette 150  $\mu$ L of the serum samples into the sample cup. Pipette 20-25 samples at a time and place the racks on the input buffer tray.
- 5) Ensure that the amount of reagents, diluent, and wash solutions are 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

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**c. Initiating a Run**

***Do not load samples on the input tray if the green light is flashing.***

***When the instrument starts, it will run the default profile on all samples unless programmed differently prior to loading.***

- 1) Once you have loaded racks onto the sample input buffer, click on the “Start” global button.
- 2) Click “Start” on the Start Conditions screen that appears. This will start the instrument.
- 3) After the tube barcode is scanned, go to the “Workplace” screen and select the “data review” tab.
- 4) To enter the patient ID, highlight the line that contains a tube number. Click the “demographics” button at the bottom of the screen.
- 5) Place the cursor in the patient ID box and scan in the barcode from the patient vial that corresponds to the tube number the sample was placed in.
- 6) Click “OK” and continue until all patient ID’s are entered.
- 7) Step 6 must be completed prior to printing results and backing up data.

**d. End of Assay**

- 1) Once results are complete, review all results from the “Data Review” screen.
- 2) Highlight all results to be printed and click the “print” global button.
- 3) Check “both” to print original result and all repeats. Click “print” to print the data.
- 4) To backup the data, insert 3 ½ inch disk into A:\ drive and highlight all samples to archive on the “data review” screen.
- 5) Click “Backup data” on the bottom of the screen. A box will appear asking how you want to save the data (Floppy or Zip, ASCII or binary) and what the file name is.
- 6) First, save the file to the floppy as an ASCII file and use the date the run was done as the file name.
- 7) Next, save the file to the zip drive as a binary file and use the date the run was done as the file name.
- 8) After all data is saved, “delete all” data from the “data review” screen.

**e. System Maintenance**

The E-170 system maintenance consists of daily, weekly, 2 week and as needed maintenance [12].

- 1) Daily maintenance (Refer to the Hitachi Modular Analytics<sup>®</sup> Operator’s Manual) should be performed at the start of each 8-hour shift, or more frequently, if necessary. It consists of cleaning the sample probe, reagent probe, sipper probes, preclean probes and all instrument surfaces. Printer paper supply along with reagent overview should be checked.
- 2) Weekly maintenance (Refer to the Hitachi Modular Analytics<sup>®</sup> Operator’s Manual) consists of cleaning mixers, reservoirs, incubator, rinse stations and fill nozzles.

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3) 2 week maintenance (Refer to the Hitachi Modular Analytics<sup>®</sup> Operator's Manual) requires liquid flow path cleaning.

### f. Special Method Notes

The system can be turned off completely for the weekend or extended holidays or when indicated by maintenance procedure or error code.

### g. Calculations

All calculations are performed by the Hitachi Mod PE<sup>®</sup> Software system using a machine-stored calibration curve.

### h. CDC Modifications

The method is run exactly as stipulated by Roche Diagnostics; CDC has introduced no modifications.

## 9. Reportable Range of Results and Dilutions

The reportable range is defined by the lower detection limit and the maximum of the master curve. For the purposes of CDC reporting, we will use a reportable range of 0.5-2000 ng/mL.

Samples with values of <10.0 ng/mL will be automatically repeated to confirm the low result.

Samples with values <0.5 ng/mL will be reported as <0.5 ng/mL. Samples with values of >2000 ng/mL are automatically diluted and reanalyzed. The result is multiplied by the appropriate dilution factor.

## 10. Quality Control (QC) Procedures

### a. Blind Quality Control

Blind QC specimens are inserted prior to the arrival of the samples in the Nutritional Biomarkers 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 three serum pools, which represent low, med and high levels of Ferritin 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 all QC results are within 2s limits and the run is accepted. If one of the three 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 three QC results is outside the 3s limit
- $2_{2s}$  Two 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

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- 10<sub>x</sub> 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 [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_LABS\Data handling\QC\Hitachi MOD PE QC](#). 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. Samples need to be analyzed within 2 hours of being placed on the instrument to minimize the effects of evaporation.

### 13. Reference Ranges (Normal Values)

Ferritin reference ranges are assay-specific. The Roche Diagnostics kit specifies expected values of 30-400 ng/mL for men and 13-150 ng/mL for women [13].

### 14. Critical Call Results ("Panic Values")

For NHANES 1999+, since data are transmitted 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

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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 email or FAX by the supervisor of any Ferritin result that is <15 ng/mL, 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 FrontEnds database is used to keep records and track specimens for NHANES 1999+. Records are kept in files on [\\cdc\project\CCEHIP\\_NCEH\\_DLS\\_NBB\\_LABS](#) 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.

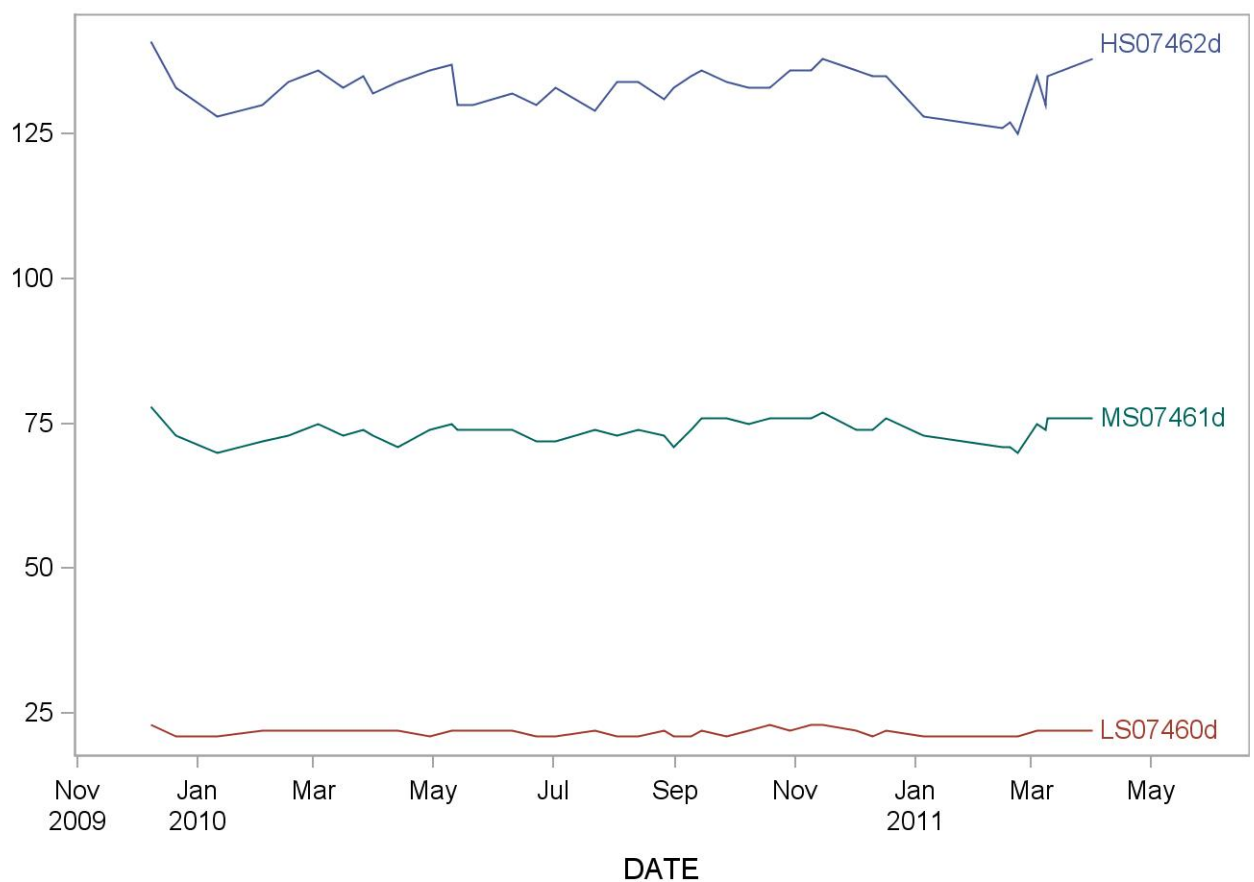
The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the 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.

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**19. Summary Statistics and QC Graphs**

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HS07462d	89	08DEC09	01APR11	131.1	3.9	2.9
LS07460d	68	08DEC09	01APR11	21.3	0.8	3.8
MS07461d	83	08DEC09	01APR11	72.8	2.2	3.0

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## **ACKNOWLEDGMENTS**

We gratefully acknowledge the contributions of Donna LaVoie and Christine Pfeiffer who assisted in evaluating this assay and preparing the manuscript for this chapter.

**Appendix (Addendum 1)**

**Addendum 1**

**Ruggedness Testing**

As this assay must be performed according to the manufacturer's specifications, none of the parameters can be altered. Therefore, ruggedness testing has not been done for this assay.