Laboratory Procedure Manual

Analyte: **Ferritin**

Matrix: **Serum**

Method: **Electrochemiluminescence immunoassay "ECLIA"**

Method No: 4046.05

Revised: November 2017

as performed by: Nutritional Biomarkers Branch (NBB)
Division of Laboratory Sciences (DLS)
National Center for Environmental Health (NCEH)

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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.

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
FERITIN_J	LBXFER	Ferritin (ng/mL)
	LBDFERSI	Ferritin (µg/L)

1. Summary of Clinical Relevance and Principle

A. Clinical Relevance

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^{3+} 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 lyosomes 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 pre-latent 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.

B. Test Principle

The method for measurement of Ferritin on the cobas® e601 is a sandwich principle with a total duration time of 18 minutes. The 1st incubation uses 10 µL of sample, a ferritin-specific antibody and a labeled ferritin-specific antibody to form a sandwich complex. The 2nd 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.

2. Safety Precautions

Consider all specimens potentially positive for infectious agents including HIV, hepatitis B and hepatitis C. We recommend that the hepatitis B vaccination series for all the analysts working with whole blood and/or serum. 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 all disposable plastic, glassware, and paper (pipette tips, vials, gloves, etc.) in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach or similar disinfectant 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 are listed in Section 6. Safety data sheets (SDSs) for all chemicals are readily accessible as hard copies in the lab. If needed, SDS for other chemicals can be viewed at http://intranet.cdc.gov/ossam/workplace-safety/safety-practices/chemical-safety/index.html or at http://www.ilpi.com/msds/index.html.

3. Computerization; Data System Management

(A) During sample preparation and analysis, samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.

(B) Calculation of Ferritin concentration is accomplished with the software on the Roche e601 and generated data are transferred to the DLS network where it is saved. The results file is imported into a database for review of the patient data, statistical evaluation of the QC data, and approval of the results. See **"SOP Computerization and Data System Management"** for a step-by-step description of data transfer, review and approval.

(C) NHANES data is transmitted electronically on a regular basis (approximately weekly for 3-week turnaround analytes). 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. NCHS makes arrangements for the abnormal report notifications to the NCHS Survey Physician.

(D) The data file and results file from the instrument workstation are typically backed up daily to a USB and/or DVD for long-term storage. This is the responsibility of the analyst under the guidance of the project lead person. Files stored on the DLS network are automatically backed up nightly by ITSO support staff.

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

(A) For best results, a fasting sample should be obtained, but fasting is not required. Centrifuge samples containing precipitate before performing the assay.

(B) Specimens for Ferritin analysis may be fresh or frozen serum or plasma. Serum specimens may be collected with regular red-top Vacutainers or tubes containing separating gel and plasma specimens may be collected with LI-, Na-heparin, K3-EDTA and sodium citrate as an anticoagulant. When sodium citrate is used, the results must be corrected by + 10 %. Samples and controls stabilized with azide or heat-inactivated samples will be rejected **[8]**.

(C) The appropriate amount of serum is dispensed into a Nalgene cryovial or other plastic screw-capped vials labeled with the participant's ID. A 500-µL sample of serum or plasma is preferable to allow for repeat analyses; a minimum volume of 150 µL is required for pipetting into the sample cup.

(D) Specimens collected in the field should be kept cold and protected from light. After processing, specimens should be frozen and shipped on dry ice by overnight mail. Once received, samples should be stored at <-20 °C until analyzed. For long term storage, specimens should always be frozen at-70 °C. Serum Ferritin is stable for 2 weeks at 2-8 °C **[9]** and 12 months at -20 °C **[10]** and it can withstand 3 freeze/thaw cycles **[9]**.

(E) Ensure that the patients' samples, calibrators and controls are at ambient temperature (20-25 °C) before measurement. Once the samples, calibrators, and controls are loaded on the analyzers, they should be measured within 2 hours because of possible evaporation effects.

(F) Specimen handling conditions are outlined in the DLS Policies and Procedures Manual. The protocol discusses collection and transport of specimens and the special equipment required. If there is more than one test of interest in the specimen and it needs to be divided, the appropriate amount of blood, serum or plasma should be transferred into a sterile Nalgene cryovial labeled with the participant's ID; avoid cross contamination.

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. 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 until the expiration date or up to 12 weeks at 2-8 °C after opening, whichever comes first. **The Ferritin reagent pack can only be stored on-board the e601 for a maximum of 6 weeks** so the reagent pack is generally removed from the instrument and stored at 2-8 °C when all samples are completed.

B. Standards Preparation

Elecsys Ferritin CalSet is supplied by Roche Diagnostics in liquid form. Store the standards at 2-8 °C until the expiration date of the kit.

C. Preparation of Quality Control Materials

1) Roche controls

Elecsys PreciControl Varia **[11]** can be used for quality control of the Elecsys Ferritin immunoassay on the e601 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 cobas® 6000 before analysis. Use Class A volumetric glassware if volumetric glassware is specified in the package insert.

To reconstitute the PC Varia, carefully dissolve the contents of one bottle of each level by adding exactly 3.0 mL of distilled water and allow to stand closed for 30 minutes. Mix carefully, avoiding the formation of foam. Transfer aliquots of the reconstituted controls into appropriately labeled empty snap-cap vials avoiding cross contamination. 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

Quality control materials for this assay are prepared in-house from blood products acquired from blood banks or from other volunteer blood donors. After screening the pools for Ferritin, the serum

is pooled to obtain the desired QC levels. All pools are filtered through gauze to remove debris before being dispensed. Serum (usually 750 µL) is aliquoted into labeled 2.0-mL Nalgene cryovials, capped, and is typically stored at <-70 °C. The QC pools are stable for at least 3 years.

The QC limits for all pools are established by analyzing duplicates of each pool for at least 20 consecutive runs.

D. Other Materials

The following materials are available from the manufacturer (Roche Diagnostics):

(1) Sample racks

(2) Sample cups (Standard)

(3) ProCell M system buffer

(4) CleanCell measuring cell cleaning solution

(5) PC/CC-Cups to prewarm ProCell M and CleanCell M

(6) ProbeWash M cleaning solution for finalization and rinsing

(7) PreClean M detection cleaning solution

(8) Assay Tip/AssayCup Combimagazine M (reaction vessels and pipette tips)

(9) WasteLiner

(10) SysClean system cleaning solution

E. Instrumentation

In the case of simple laboratory instrumentation (e.g., pipettes, vortex mixer, analytical balance, etc.) a product listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed.

(1) Roche Cobas® 6000 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

For commercial kit assays, calibration procedures recommended by the manufacturer are followed.

Roche Diagnostics standardized this assay against previous Elecsys Ferritin assays that were standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) “Reagent for Ferritin (human liver)” 80/602. Recovery studies, including a published study **[12]**, 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.

Every Elecsys Ferritin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys

Ferritin CalSet. The Ferritin CalSet lot-specific calibrator values are encoded in the barcode as well as electronically available and must be entered in the analyzer prior to use. Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 2 months (8 weeks) when using the same **reagent lot**
- every 7 days if using the same **reagent kit**
- as required: e.g. if quality control findings are outside the specified limits

Please refer to the Roche *Cobas® 6000 Operator's Manual* and the **"SOP Cobas® 6000 Calibration and QC"** for additional details.

Calibration verification is conducted at least twice a year using international reference materials. For details, see **4046_SOP Calibration and Calibration Verification FER**.

The NIBSC Code 94/572 3rd International Standard for Ferritin, Recombinant (**NIBSC 94-572 Serum ferritin recombinant.pdf**) is available for calibration verification **[13]**.

Calibration can also be verified by running the Elecsys Ferritin CalCheck as unknowns.

The laboratory participates in the College of American Pathologists (CAP) external 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.

When possible, the laboratory may also participate in other proficiency testing programs such as the UK NEQAS Haematinics survey (twice a year).

For general information on the handling, analysis, review, and reporting of proficiency testing materials, see **"NBB_SOP Proficiency Testing Procedure"**.

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

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

(1) Allow Calibrators, QC and patient samples to reach ambient temperature.

(2) 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.

(3) Make sure the analyzer and/or tests required are not masked.

(4) Check to see if calibration is required for the tests that will be run.

(5) If running the same tests on all samples, go to the "Start" global button and set the "default profile".

(6) Be sure to clear all previously programmed samples from the Data Review screen after backing up the data.

(7) Perform the required maintenance on the e601 system.

B. Instrument Maintenance

The e601 system maintenance consists of daily, weekly, 2 week, 3 months and as needed maintenance **[14]**.

(1) Daily maintenance should be performed at the start of each 8-hour shift, or more frequently, if necessary.

(2) For additional maintenance requirements, refer to the instrument maintenance logs. For detailed, step by step instructions, refer to the Cobas® 6000 Operator's Manual.

C. Preparing a Run

One run is defined as 100 samples or less.

Each run must contain 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 Ferritin QC pools must be run in duplicate.

NOTE: Before starting a new run, backup all previous test results and clear the "data review" screen.

(1) Thoroughly mix all calibrators, QC and patient samples before pipetting. Visually check for any unusual sample volume, specimen color or debris/precipitate.

(2) Prior to loading samples on the instrument, 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.

(3) For a calibration run, use **black** calibrator racks. Open the barcoded calibrators and place them in an unassigned **black** calibrator rack or pipet 150 µL of each calibrator into a sample cup placed in the assigned positions in a **black** calibrator rack. When calibration is completed, the results will be printed.

(4) To run QC, use the **white** QC racks. For Roche and CDC QC, pipette 150 µL of each nonbarcoded QC into a sample cup and place atop the tube in the assigned control position of the **white** QC racks. When QC is completed, the results will be automatically printed.

(5) To run patient samples, use the **gray** sample racks. Place empty sample cups onto barcode labeled 13 X 75 tubes in **gray** sample racks and pipette 150 µL of the serum samples into the sample cup. Pipette 20-25 samples at a time and immediately place the racks on the input buffer tray. **Gray racks with yellow stickers are for urine samples only**. Patient results do not print until requested.

D. 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 the calibrator, control or sample racks are loaded on the input tray, they should be measured within 2 hours because of possible evaporation effects. Calibration and QC checks must be completed before pipetting patient samples.

(2) For detailed, step-by-step instructions, refer to **"SOP Cobas® 6000 Operation"** or the *Cobas® 6000 Operator's Manual*.

E. Processing and Reporting a Run

(1) The Cobas® 6000 Control Module is used to review data and check for samples that need to be diluted or repeated for confirmation. The LIMS database is used for an additional level of data review by the analyst, project lead, QA officer, and supervisor and for data reporting.

(2) For more detailed information, refer to Section 3 and the **"SOP Computerization and Data System Management"**.

F. Special Method Notes

The system can be turned off for the weekend or extended holidays or when indicated by maintenance procedure or error code. Refer to the *Cobas® 6000 Operator's Manual* for instructions.

G. Calculations

All calculations are performed by the Cobas® 6000 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 (AMR – Analytical Measurement Range)

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 <15.0 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 1:50 with Elecsys Diluent Universal and reanalyzed via the rerun function. The instrument automatically takes the dilution into account when calculating the sample concentration. Dilutions done manually must be multiplied by the correct dilution factor. The maximum acceptable dilution would give a diluted sample result >40 ng/mL **[8]** before multiplying by the dilution factor. When possible, avoid small volume pipetting and minimize use of serial dilutions when generating diluted sample results.

10. Quality Control (QC) Procedures

A. Blind Quality Controls

Blind QC specimens can be inserted into the mix of patient specimens. These QC specimens are generally prepared at two levels that would be encountered in 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.

Alternatively, open label blind QC specimens can be used where the analyst knows that the sample is a blind QC, but they do not know what pool the sample is from. Open label blind QCs are only used if one can choose from at least 6 different pools and the analyte concentrations are similar to those found in patient samples.

B. Bench Quality Controls

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

Three QC pools per run with two or more QC results (replicates) per pool:

(1) If all three QC run means are within 2 S_m limits and individual results are within 2 S_i limits, accept the run

(2) If 1 of the 3 QC run means is outside a 2 S_m limit – reject run if:

a) 1 3S Rule—Run mean is outside a 3 S_m limit or

b) 2 2S Rule—Two or more of the three run means are outside the same 2 S_m limit or

c) 10 X-bar Rule—Current and previous nine run means are on the same side of the characterization mean

(3) If one of the six QC individual results is outside a 2 S_i limit – reject run if:

a) Outlier – One individual result is beyond the characterization mean ± 4 S_i or

b) R 4S Rule—Two or more of the within-run ranges in the same run exceed 4 S_w (i.e. 95 percent range limit)

S_i = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).

S_m = Standard deviation of the run means (the limits are shown on the chart).

S_w = Within-run standard deviation (the limits are not shown on the chart).

The QC results are checked after each run using of a multi-rule quality control program **[15]**. 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 are reevaluated periodically. When necessary, limits are updated to include more runs.

While a study is in progress, QC results are stored in a LIMS database. For runs that are not imported into the database (i.e., R&D, troubleshooting, research-type runs), QC results are stored electronically in the analyte-specific folder on the DLS network.

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

(A) Check to make sure that the hardware is functioning properly.

(B) Recalibrate the instrument.

(C) Rerun Bench QC; run Roche QC

(D) Analyze reference material.

(E) Call the Roche “hotline” or service engineer.

(F) Do not report analytical results for runs not in statistical control.

(G) 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.

12. Limitations of Method; Interfering Substances and Conditions

(A) In patients receiving therapy with high biotin doses (i.e. >5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

(B) Do not use samples and controls stabilized with azide or heat-inactivated samples **[8]**.

(C) For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

(D) Assay needs to be performed within 2 hours of the samples being placed on board the instrument to minimize the effect of evaporation.

13. Reference Ranges (Normal Values)

The Roche Diagnostics kit specifies expected values of 30-400 ng/mL for men and 13-150 ng/mL for women **[16]**.

Reference ranges for the U.S. population generated with the Ferritin Tina-quant assay for NHANES 2003-2006 and published in the Second Nutrition Report **[17]** are shown below (also available by population subgroups):

Serum ferritin – children 1-5 y:	6.68-77.5 ng/mL (2.5th -97.5th percentile; n = 1,482)
Serum ferritin – women 12-19 y:	4.64-103 ng/mL (2.5th -97.5th percentile; n = 1,991)
Serum ferritin – women 20-39 y:	4.67-176 ng/mL (2.5th -97.5th percentile; n = 1,780)
Serum ferritin – women 40-49 y:	4.38-264 ng/mL (2.5th -97.5th percentile; n = 759)

14. Critical Call Results (“Panic Values”)

Ferritin results <15 ng/mL or >400 ng/mL are indicative of iron deficiency or iron overload and require follow-up (repeat analysis for confirmation of ferritin level). Since survey data are transmitted several times

weekly to Westat, abnormal reports are automatically forwarded to the NCHS survey physician for follow-up. For smaller, non-NHANES studies, abnormal values are identified to the study principal investigator. Emails sent concerning abnormal results are maintained by the supervisor for the duration of the study. Most of these studies are epidemiological in nature.

15. Specimen Storage and Handling during Testing

Specimens are allowed to reach room temperature during preparation. After analysis, the unused portion of the patient specimen is then returned to frozen storage (typically ≤-70 °C) as soon as possible.

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)

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 Excel file, generally through electronic mail or via ftp site.

For NHANES 1999+, all data are reported electronically weekly to Westat who then transfer the results 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

A LIMS database is used to keep records and track specimens for NHANES 1999+. If analyses are performed for smaller, non-NHANES studies, records may be kept in Excel files on the DLS network.

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 are retained for at least 1 year after results have been reported and may then be returned or discarded at the request of the principal investigator. Very little residual material will be available after NHANES analyses are completed, however residual serum is retained for at least 2 year after results have been publicly released; at that point, samples with sufficient volume (>0.2 mL) are returned to NHANES and samples with insufficient volume may be 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 on the vial is scanned by a barcode reader and used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the result file is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping records of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. In general, these are documented using codes in the LIMS.

19. Method Performance Documentation

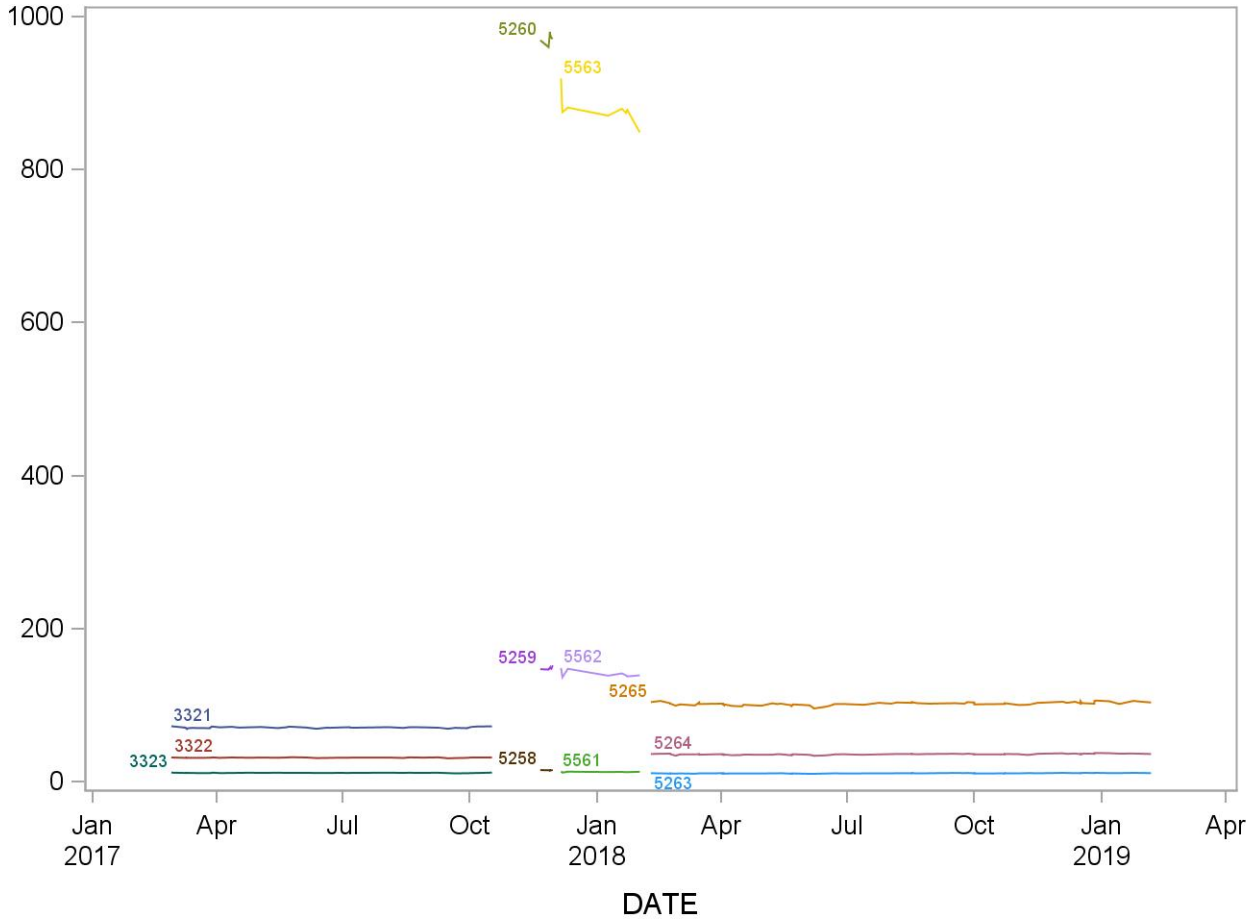
Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in Appendix A of this method documentation. The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.

20. Summary Statistics and QC Charts

Please see following pages.

2017-2018 Summary Statistics and QC Chart for Ferritin(ng/mL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3321	34	27FEB17	17OCT17	70.826	0.893	1.3
3323	34	27FEB17	17OCT17	11.434	0.214	1.9
3322	34	27FEB17	17OCT17	31.337	0.314	1.0
5260	6	21NOV17	30NOV17	971.583	7.031	0.7
5258	6	21NOV17	30NOV17	14.950	0.221	1.5
5259	6	21NOV17	30NOV17	148.417	1.985	1.3
5563	8	06DEC17	01FEB18	878.188	19.433	2.2
5561	8	06DEC17	01FEB18	12.681	0.469	3.7
5562	8	06DEC17	01FEB18	141.000	4.559	3.2
5265	62	09FEB18	06FEB19	101.865	2.070	2.0
5263	62	09FEB18	06FEB19	10.911	0.307	2.8
5264	62	09FEB18	06FEB19	35.819	0.783	2.2



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Appendix A: Method Performance Documentation

Accuracy compared to Reference Material - fill in yellow shaded cells

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name:	Ferritin
Method #:	4046
Matrix:	Serum
Units:	ng/mL
Reference material:	**NIBSC 94/572**
Analyte:	**FER**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	1575	1730	1629	1623	1572	1630	1649	74.93	4.55	4.7
	2		1775	1710	1669	1522	1627				
Level 2	1	630	665	659	672	641	651	664	15.46	2.33	5.4
	2		677	689	677	644	664				
Level 3	1	126	140	138	136	133	136	137	2.37	1.73	8.8
	2		138	140	136	135	139				
Level 4	1	31.5	35.7	34.9	34.9	34.2	35.6	35.1	0.63	1.79	11.6
	2		35.9	35.3	35.2	34.1	35.6				
Level 5	1	6.3	6.85	6.95	6.88	7.15	7.18	7.02	0.20	2.90	11.4
	2		6.87	7.06	6.70	7.19	7.37				

Precision - fill in yellow shaded cells

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Ferritin
Method #:	4046
Matrix:	Serum
Units:	ng/mL
Analyte:	**FER**

Low QC material LS13460

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1 - 5/3/17	11.5	11.6	11.55	0.0025	0.0025	266.805
2 - 5/15/17	11.5	11.8	11.65	0.0225	0.0225	271.445
3- 5/18/17	11.8	11.7	11.75	0.0025	0.0025	276.125
4 - 5/22/17	11.6	11.3	11.45	0.0225	0.0225	262.205
5 - 5/23/17	11.5	11.6	11.55	0.0025	0.0025	266.805
6 - 6/5/17	11.4	11.6	11.50	0.01	0.01	264.5
7 - 6/6/17	11.3	11.8	11.55	0.0625	0.0625	266.805
8 - 6/12/17	11.4	11.3	11.35	0.0025	0.0025	257.645
9 - 6/20/17	11.2	11.6	11.40	0.04	0.04	259.92
10 - 6/21/17	11.2	11.5	11.35	0.0225	0.0225	257.645
Grand sum	230.2	**Grand mean**	11.51			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.38	0.038	0.194935887	1.69
Between Run	0.298	0.033111111	0	0.00
Total	0.678		0.194935887	**1.69**

High QC material HS13462

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1 - 5/3/17	71.8	71.2	71.50	0.09	0.09	10224.5
2 - 5/15/17	69.6	70.4	70.00	0.16	0.16	9800
3- 5/18/17	73.0	70.0	71.50	2.25	2.25	10224.5
4 - 5/22/17	72.2	70.4	71.30	0.81	0.81	10167.38
5 - 5/23/17	72.0	71.9	71.95	0.0025	0.0025	10353.605
6 - 6/5/17	70.4	71.0	70.70	0.09	0.09	9996.98
7 - 6/6/17	69.9	72.2	71.05	1.3225	1.3225	10096.205
8 - 6/12/17	68.8	69.3	69.05	0.0625	0.0625	9535.805
9 - 6/20/17	69.0	72.4	70.70	2.89	2.89	9996.98
10 - 6/21/17	70.5	70.1	70.30	0.04	0.04	9884.18
Grand sum	1416.1	**Grand mean**	70.805			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	15.435	1.5435	1.242376754	1.75
Between Run	13.1745	1.463833333	0	0.00
Total	28.6095		1.242376754	**1.75**

Stability - fill in yellow shaded cells

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions

Describe condition: three times frozen at -70°C and three times thawed (4 hrs) at room temperature (3 freeze-thaw cycles)

Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)

Describe condition: QC material stored at room temperature for 6 hrs, refrozen and thawed

Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler

Describe condition: QC material aliquoted into sample cups and stored on instrument for 2 hrs at room temperature before analysis

Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis

Describe condition: QC material stored at -70°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name:	Ferritin
Method #:	4046
Matrix:	Serum
Units:	ng/mL
Analyte:	**FER**

Low QC material LS13460							2015	2017
8/1/2017	**Initial measurement**	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	11.6	11.63	11.6	11.7	11.6	12.11	11.34	11.6
Replicate 2	11.74	11.39	11.74	11.41	11.74	12.43	10.91	11.74
Replicate 3	11.6	11.58	11.6	11.75	11.6	12.4	10.81	11.6
Mean	11.65	11.53	11.65	11.62	11.65	12.31	11.02	11.65
% difference from initial measurement	--	**-1.0**	--	**-0.2**	--	**5.7**	--	**5.7**

High QC material HS13462							2015	2017
8/1/2017	**Initial measurement**	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	70.77	71.94	70.77	71.15	70.77	72.99	66.55	70.77
Replicate 2	71.5	71.29	71.5	70.16	71.5	74.52	68.01	71.5
Replicate 3	71.79	71.36	71.79	71.13	71.79	74.06	66.58	71.79
Mean	71.35	71.53	71.35	70.81	71.35	73.86	67.05	71.35
% difference from initial measurement	--	**0.2**	--	**-0.8**	--	**3.5**	--	**6.4**

LOD, specificity and fit for intended use - fill in yellow shaded cells

Method name:	Ferritin		
Method #:	4046		
Matrix:	Serum		
Units:	ng/mL		
	Kit assay is FDA approved- LOD and interference information are provided by the manufacturer		
Analytes	**Limit of Detection (LOD)**	**Interferences successfully checked in at least 50 human samples**	**Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use**
Ferritin	0.5	Samples should not be taken from patients receiving therapy with high biotin doses until at least 8 hours following the last administration.	yes