Laboratory Procedure Manual

Analyte: **Ferritin**

Matrix: **Serum**

Method: **Electrochemiluminescence immunoassay "ECLIA"**

Method No: 4046.07

Revised: January 2023

as performed by: Nutritional Biomarkers Branch (NBB)
Division of Laboratory Sciences (DLS)
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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.

Images are included in this document as visual aids for certain topics. They are intended to be representative images only and should not be construed as absolute references. Discrepancies between the images in this document and the actual application design are not a cause for revisions to this document.

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_L	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 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.

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. Additional information on hazard identification, risk evaluation and risk mitigation for this method can be found in the method risk assessment document.

3. Computerization; Data System Management

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.

Calculation of Ferritin concentration is accomplished with the software on the Roche e601, and data files are transferred and saved on DLS network. 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 **Appendix B_C "JA-4046-DR-01-Computerization and Data System Management"** for a step-by-step description of data transfer, review, and approval.

The data files from the instrument workstation are routinely backed up to a USB for long-term storage. 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 kept frozen during "in processing", which is typically completed within less than 4 hours and then stored at ≤-50°C for up to 15 business day until transferred to the testing laboratory for longer storage at deep frozen conditions, typically around -70°C (-50°C to -90°C). Serum ferritin is stable for 2 weeks when refrigerated (+2°C to +8°C) **[9]** and 12 months when stored frozen 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 (+15°C to +30°C) before measurement. Once the samples, calibrators, and controls are loaded on the analyzers, they should be measured within 2 hours to avoid 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.

(G) The criteria for unacceptable specimens are insufficient sample volume (<150 µL) for at least one analysis, suspected contamination such as leaking, or damaged sample container. These samples are assigned an appropriate comment code and/or description and are set "no reportable (code 98).

(H) A series of standard comment codes are available in the STARLIMS database to identify any issues related to sample quality. These codes can be used, along with text descriptions, to document why a result was not reported (specimen rejection) or that a result should be interpreted with caution based on the sample quality.

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 to ensure complete availability of the microparticles. Bring the cooled reagents to approximately 20°C (45 minutes at ambient temperature) and open the lids slightly before placing on the reagent disk of the analyzer. The reagent kit is stable under refrigerated condition (+2°C to +8°C) until the expiration date or up to 12 weeks after opening, whichever comes first. **The Ferritin reagent pack can only be stored on-board the e601 for a maximum of 6 weeks.** Therefore**,** the reagent pack is generally removed from the instrument and stored refrigerated (+2°C to +8°C) when all sample analyses are completed. This extends the stability of the reagent pack to 60 days when stored for shorter periods on the analyzer.

B. Standards Preparation

Elecsys Ferritin CalSet (contains STD1 and STD2 calibrators) is supplied by Roche Diagnostics in liquid form and ready to use. Store the standards refrigerated (+2°C to +8°C) until the expiration date of the kit.

C. Preparation of Quality Control Materials

1) Roche QC Materials

Elecsys PreciControl Varia **[11]** are used for the daily quality control checks of the Elecsys Ferritin immunoassay on the e601 Analyzer. This is a lyophilized control serum based on human serum matrix at three concentrations. 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 each of the three bottles by adding exactly 3.0 mL of -deionized water (water with a resistivity of at least 18 MΩ); allow to stand closed for 30 minutes to reconstitute. Mix carefully, avoiding foam formation. 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 refrigerated (+2°C to +8°C) are stable for up to 3 days. Ensure the controls are at ambient temperature (+15°C to +30°C) before use.

2) CDC QC pools

Additional QC 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 ultra-low temperatures -typically around -70°C (-50°C to -90°C). The QC pools are stable for at least 3 years. The CDC QC pools are analyzed a few times per month to assess long-term trends; they are not used on a daily basis as quality control checks.

For more detailed information on the preparation of QC materials, homogeneity testing, and characterization refer to **SOP "NBB-OC-LABOP.01.01 QC Materials".**

3) QC Set-up on the Analyzer

QC information must be installed on the instrument system before use when a new lot number of QC is needed. Roche QC can be added manually or by using the Cobas link. Non-Roche QC are added by going to "QC/Install/Add". After adding new QC these need to be activated for the test and assigned to a white QC rack and position.

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 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 micropipette 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., no 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

Calibration verification is conducted at least twice a year using international reference materials. For details, see **Appendix B_A: "JA-4046-G-01-Calibration and Calibration Verification"**

The NIBSC Code 94/572 3rd International Standard for Ferritin, Recombinant (**NIBSC 94-572 Serum ferritin recombinant.pdf**) is available for calibration verification **[13]**. Reference materials are stored deep frozen (-50°C to -90°C).

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

Details about our proficiency testing (PT) activities can be found in the proficiency testing form. The laboratory participates in two College of American Pathologists (CAP) external proficiency testing programs: (CAP) K Ligand survey (3 times per year) and LN5 Calibration Verification Ligand survey (2 times per year). When possible, we also participate in other external proficiency testing program such as the UK NEQAS Haematinics survey

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, QCs, and patient samples to reach ambient temperature (+15°C to +30°C).

(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 (Cobas 6000 Clinical Analyzer).

B. Instrument Maintenance & Function Checks

The e601 system maintenance (Cobas 6000 Clinical Analyzer) consists of daily, weekly, monthly, quarterly, and as needed maintenance **[14]**.

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

- Daily maintenance and function checks include running the pre-programmed daily maintenance (***Daily pipe***), checking the deionized water (diH_2O) supply to the instrument water tank; manually cleaning the sample probes using diH_2O moistened gauze pads; cleaning reagent probes, sipper probes, and pre-wash probes using gauze pads moistened with alcohol followed by diH_2O, manually cleaning cell rinse nozzles and the drain port with diH_2O; maintaining printer paper supply and checking reagent levels; The "green rack" should be run at the end of each day after all sampling is completed and before the "***Sleep pipe***" to bring the module in standby mode.

- Weekly maintenance consists of cleaning ProCell and CleanCell nozzles, replacing reservoir bottles; and cleaning reservoir positions, mixers, separation stations, incubator, Assay cup vortex mixer, microbead mixer and rinse stations. An ***E module pipe*** should be done on the e601 weekly or before use if 24 hours have lapsed since the last use. The entire Cobas® 6000 should be powered "Off/On" typically once per week.

- The 2-week maintenance requires liquid flow path cleaning on the e601.

- Monthly checks include cleaning the diH_2O tank, all air filters (4), and filter for rack sampler unit, water bath, KCl, and detergent aspiration filters.

- As needed maintenance also includes cleaning of aspiration tubes, bottle stands, reagent disk solid waste compartments and all instrument surfaces.

(2) For additional maintenance requirements, refer to **Appendix B_B "JA-4046-I-01-Instrument Maintenance & Function Checks".**

C. Preparing a Run

A run is defined as 100 patient samples or less and 2 sets of FER Roche QC pools analyzed with patient samples at the beginning and at the end of each run.

(1) Mix all calibrators, QCs, and patient samples thoroughly before pipetting. Visually check for any unusual sample volume, specimen color or debris/precipitate. Ensure barcodes are facing the open slot in the racks.

(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. Nonbarcoded calibrators must be pipetted (150 µL) into sample cups and placed in their assigned positions in "**black**" calibrator racks. When calibration is completed, the results will be printed.

(4) To run QC, use the "**white**" QC racks. If using Roche barcoded QC, open the QC's and place them in a "**white**" unassigned rack or assign positions in white QC racks and pipet into sample cups. For CDC QC, pipette 150 µL of each non-barcoded QC into a sample cup and place in the assigned control position of the "**white**" QC racks. When the instrument is started, it will automatically run the correct tests on the preprogrammed QC and print the results.

(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.

(6) Pipette 20-25 samples at a time and immediately place the racks on the input buffer tray. Patient results do not print until requested.

(7) Avoid loading racks on the input tray if the instrument green light is flashing or turned off

D. Initiating a Run

Note: Backup all previous data from the instrument before starting a new run. Check the default profile before starting a run.

(1) Instrument starts with running default profile on all samples unless programmed differently prior to loading.

(2) 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.

(3) Calibration and QC checks must be completed before pipetting patient samples. Verify calibration and QC results print outs for run acceptability criteria.

(4) To run patient samples, load the "**gray"** (serum) sample racks onto the sample input buffer tray and click on the "**Start**" global button.

(5) On the Start Screen, click **"Start"** for sampling on the instrument.

(6) Instrument completes tube barcode scanning; next click data review on **"Workplace"** screen.

(7) Highlight the line with tube barcode number and click the **"demographics"** to match the tube barcode position to the correct sample vial. Place the cursor in the vial ID box to scan patient vial barcode.

(8) Click **"OK"** and continue until all patient ID's are entered, print results and back-up the data. All ID's must be entered prior to printing results and backing up the data.

(9) Repeat all samples that require confirmation or dilutions.

E. Processing and Reporting a Run

The Cobas® 6000 Control Module is used to review data and check for samples that need to be diluted or repeated for confirmation.

(1) Once results are complete, results are reviewed 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 repeat results. Click **"print"** to print the data.

(4) To back-up the data, the instrument must be in *Stand By.* In case instrument is not in *Stand By*, select the global **"Start"** button; in Rack Reception mode box select "**Change;"** deselect the Rack Reception Mode box and click **"OK".** The instrument will finish all samples and washes before going into Stand By.

(5) Upon instrument return to Stand-by mode, highlight samples that need to be backed-up to the instrument USB drive. Click "Backup data"; a box appears for "Save the data" (*Floppy or Backup Media; ASCII or binary*) with appropriate file name. If more than one run is done/day, file names as "a", "b" "c", etc. with appropriate dates are assigned.

(6) From instrument USB drive import data on network folder for transfer into STARLIMS database for further data review (analyst, project lead), approval and reporting (QA officer and supervisor). For details refer to **Appendix B_C "JA-4046-DR-01-Computerization and Data System Management"**.

(7) The data files can be backed up or printed from the Print View screen while system is in Stand-by or Operation mode (these files auto delete when the analyzer is put into Sleep mode after back-up/print but can still be accessed in Data view before next run

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 **Appendix B_B "JA-4046-I-01-Instrument Maintenance & Function Checks".**

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

As part of each run three levels of manufacturer QC are analyzed in duplicate and the manufacturer limits are used as run judge for this assay. However, twice a month in-house bench QC pools are analyzed in duplicate to assess potential assay shifts or trends.

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 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.

After a run is completed, used blind QC are removed from the run, marked with a black dot on the cap to indicate that the vial has been thawed, and returned to the blind QC box. This helps to identify which vials have been used. If a run needs to be repeated, the same blind QC can be inserted as in the initial run.

The use of blind QCs is optional but encouraged. Blind QCs are used in this method as a supplementary tool to assist in monitoring accuracy, precision, and aid in detecting errors; these are not used as part of the primary control procedures to determine if a run is out of control Bench Quality Controls.

B. Bench Quality Controls

Bench QC specimens are prepared from a minimum of 2 pools that represent low and high levels of FER. This assay typically uses 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.

The QC results are checked after each run using of a multi-rule quality control program **[15]** based their characterization data, namely: the pool mean; the pooled within-run standard deviation associated with individual QC results measured in the same run (S_w); the standard deviation associated with individual QC results (S_i); and the standard deviation associated with run mean QC results (S_m). QC rules have been designed to accommodate the use of 1–3 different QC pools during a run, the use of 1–2 measurements of each pool per run, and as many instruments as needed. These QC rules are described in the DLS Policies and Procedures Manual and a relevant selection applicable to this assay is shown below. The system is declared “in control” if all individual QC results are within 2S limits; the run is accepted. If not,

then the rules shown below are applied and the run is rejected if any condition is met; the run is declared “out of control”

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)

Abbreviations:

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).

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. The SAS QC program is used to monitor the QC performance over time for potential shifts, trending, or changes in assay precision. For assays performed routinely, quarterly statistics (mean, SD, CV) are calculated for each pool and compared to the characterization target values. For assays performed infrequently, statistics are calculated at least annually. As more QC data become available (covering multiple lots of reagents, multiple analysts, etc.), the initial QC limits can be reevaluated and updated. QC limits can also be reevaluated and updated as a result of a non-conforming event when the assay shows a higher than expected out of control rate and the root cause investigation does not reveal a correctable course of action to bring the assay back into control. This needs to be documented by a CAPA in STARLIMS. While, a study is in progress, QC results are stored in the STARLIMS 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. At the conclusion of studies complete QC records are prepared and submitted as a study QC report in STARLIMS for review by the laboratory chief, branch chief, and a DLS statistician.

C. Sample QC Criteria

Sample QC is set of criteria used to evaluate the quality of individual test result within run, and to evaluate the quality of the calibrators associated with the run. In addition to the sample QC criteria set forth in the DLS Policies and Procedures Manual that pertain to the reportable range of concentration results and calibration curves, sample QC criteria are also established for method-specific concentration and non-concentration data associated with an individual result.

The method-specific concentration and non-concentration parameters identified for sample QC evaluation, along with their associated thresholds and flagging protocols ('Pass', 'Check', 'Warn', 'Fail') are maintained and updated in the STARLIMS database, and sample QC assessment is performed and documented as part of run review process. A sample QC result flagged as 'Fail' should not be reported. A sample QC result flagged as 'Warn' or 'Check' should be reviewed both by the analyst and supervisor to determine if the quality of the result is suitable for reporting. Results that are flagged during sample QC evaluation may also be assigned one of a series of standard comment codes available in the STARLIMS database to identify the nature of the sample QC flag.

To assess the sample specific data quality, the following parameters are subject to sample QC evaluation in this method.

- On Cobas e601 instrument, check calibration for pass/fail
- In STARLIMS database, check runs for:
    - Measurable range (≥ 0.5 to ≤ 2000 ng/mL)
    - Results > measurable range (>2000 ng/mL) (fail; code 26); repeated after auto dilution (pass; code 97)
    - Results >15 ng/mL to ≤400 ng/mL (pass; code 0); no action needed
    - <LOD (LOD: 0.5 ng/mL); repeat/confirm (code 37)
    - Results ≥LOD and <15 ng/mL (incomplete); repeat/confirm (code 33)
    - Results >400 and ≤2000 ng/mL (incomplete); repeat/confirm (code 33)
    - Results null or 0 (fail, code 26); repeat/confirm
    - Delta difference for repeat results should be ≤15%; otherwise, repeat/confirm
    - Specimen volume less than expected for test (code 21); set no reportable (code 98)
    - Check and update results for appropriate comment codes; e: g., not enough specimen for repeat analysis (code 22; set no reportable); lab error, spills, contamination etc. (code 23; set no reportable); or instrument error/failure (code 24; repeat analysis)

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

For initial steps to investigate QC failures see **Appendix B-C "JA-4046-DR-02-Out-of Control Corrective Action".** Additional steps are provided as a general guideline for identifying possible problems resulting in "out of control" values for QC materials. The troubleshooting process should be done in consultation with the supervisor and may involve additional experiments beyond what is indicated below.

(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, respectively, and require follow-up (repeat analysis for confirmation of ferritin level). Since survey data are transmitted approximately weekly to WESTAT, abnormal reports are automatically forwarded to the NCHS survey physician for follow-up. For smaller, non-NHANES studies, abnormal values may be identified to the study principal investigator, depending on specific study arrangement. 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 ambient temperature (+15°C to +30°C) during preparation. After analysis, the unused portion of the patient specimen is then returned to deep frozen storage, typically around -70°C (-50°C to -90°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)

NHANES data is transmitted electronically on a regular basis 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 For smaller studies, hard copies of a data report are sent, as well as the results in electronic format.

Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, generally through electronic mail or via ftp site.

Data are transmitted via the CLIA Director, Division of Laboratory Sciences, NCEH, CDC after review by the Laboratory Supervisor, Branch Chief, and a CDC Statistician.

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

This protocol does not involve referral of specimens for testing the analytes of this method at another laboratory.

A STARLIMS 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 years 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 at ultra-low temperatures typically around -70°C (-50°C to -90°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 STARLIMS.

19. Method Performance Documentation

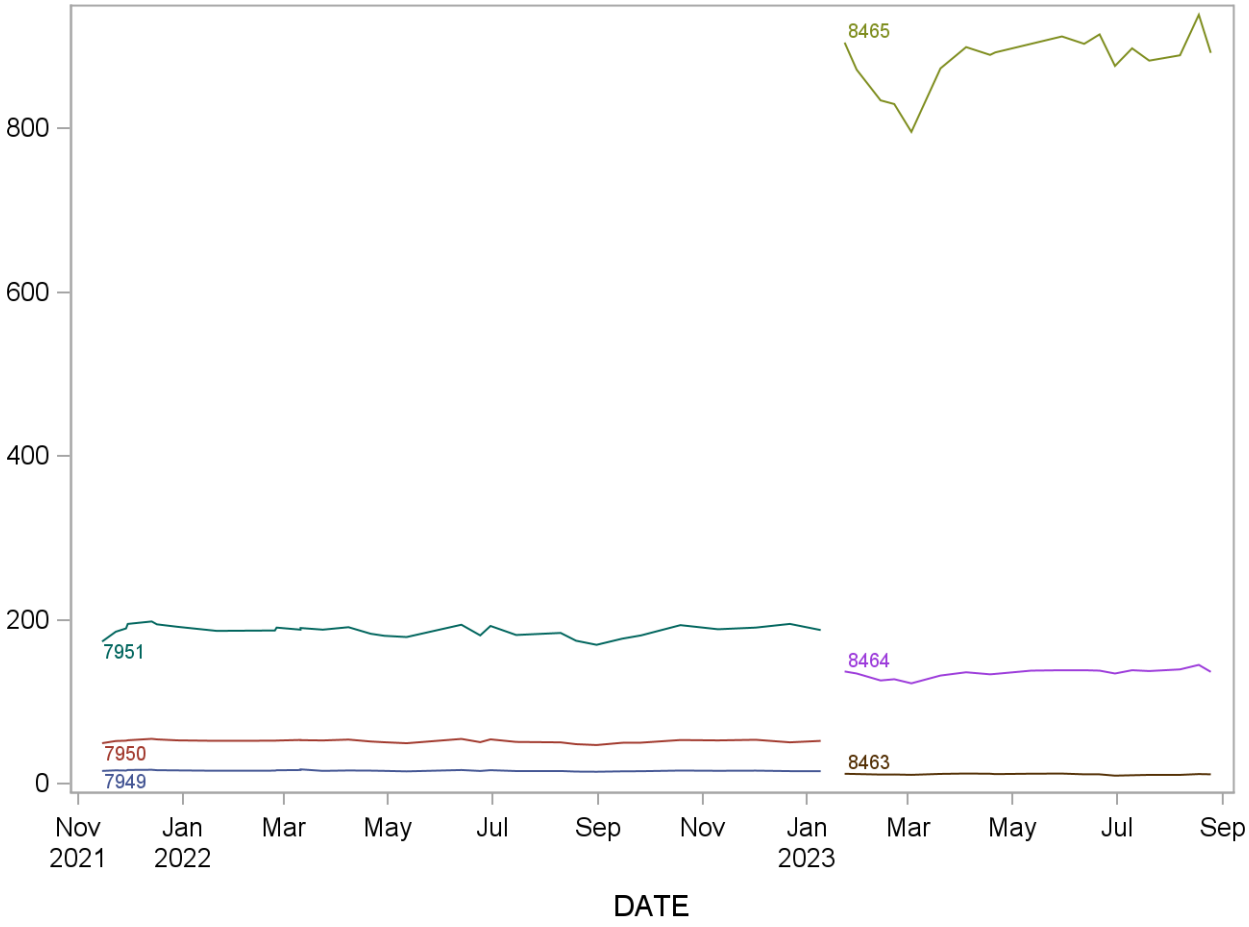
Method performance documentation for this method including accuracy, precision, sensitivity, specificity, and stability is provided in **Appendix A** of this method documentation. The approval of this procedure by the Branch chief and CLIA Director denote that the method performance is fit for the intended use of the method.

20. Summary Statistics and QC Graph

Please see following page.

August 2021 – August 2023 Summary Statistics and QC Chart
LBXFER (Ferritin(ng/mL))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
7951	31	15NOV21	09JAN23	186.484	7.023	3.8
7949	31	15NOV21	09JAN23	15.908	0.662	4.2
7950	31	15NOV21	09JAN23	51.931	1.881	3.6
8465	19	23JAN23	25AUG23	884.053	33.393	3.8
8463	19	23JAN23	25AUG23	11.448	0.717	6.3
8464	19	23JAN23	25AUG23	135.132	5.241	3.9



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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								Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)	
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 and 4 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	2019
8/1/2017 10/31/19	**Initial measurement**	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**2015 measurement**	**Long-term stability**	**Long-term**
Replicate 1	11.6	11.63	11.6	11.7	11.6	12.11	11.34	11.6	12.35
Replicate 2	11.74	11.39	11.74	11.41	11.74	12.43	10.91	11.74	12.49
Replicate 3	11.6	11.58	11.6	11.75	11.6	12.4	10.81	11.6	12.28
Mean	11.65	11.53	11.65	11.62	11.65	12.31	11.02	11.65	12.37
% difference from initial measurement	--	**-1.0**	--	**-0.2**	--	**5.7**	--	**5.7**	**12.3**

High QC material HS13462							2015	2017	2019
8/1/2017 10/31/19	**Initial measurement**	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**2015 measurement**	**Long-term stability**	**Long-term**
Replicate 1	70.77	71.94	70.77	71.15	70.77	72.99	66.55	70.77	73.58
Replicate 2	71.5	71.29	71.5	70.16	71.5	74.52	68.01	71.5	73.67
Replicate 3	71.79	71.36	71.79	71.13	71.79	74.06	66.58	71.79	76.79
Mean	71.35	71.53	71.35	70.81	71.35	73.86	67.05	71.35	74.68
% difference from initial measurement	--	**0.2**	--	**-0.8**	--	**3.5**	--	**6.4**	**11.4**

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

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

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**

Kit assay is FDA approved- LOD and interference information are provided by the manufacturer

Appendix B: APM Job Aids

A. General:

JA-4046-G-01-Calibration and Calibration Verification

This assay has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against NIBSC 80/602, 1st IS Ferritin, Human liver, 1981. Roche states that over-recovery of the 3rd IS (94/572) is ~13-16% and is for informational purposes only. All reagents are supplied by Roche Diagnostics in a ready-for-use unit. Store the reagent kit upright to ensure complete availability of the microparticle; use class A glassware; use volumetric glassware if specified in the package insert.

a) Calibration

Every Elecsys Ferritin reagent set has a barcoded label containing the specific information for calibration of the specific reagent lot. The predefined master curve is adapted to the analyzer using the relevant Roche **Ferritin CalSet calibrator.** The calibrators (STD1 & STD2) are stable when refrigerated (+2°C to +8°C) for up to 12 weeks after opening or up to stated expiration date. Every Elecsys Ferritin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. 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

Calibrators:

- **STD 1 – Ferritin CalSet – bottle 1 (ng/mL)- liquid, ready to use**
- **STD 2 – Ferritin CalSet-bottle 2 (ng/mL) – liquid, ready 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
- After 7 days (when using the same reagent kit on the analyzer)

The reagents (M, R1 and R2) have been assembled into a ready for use unit that cannot be separated. The reagents are stable for 6 weeks on the Cobas e601 or until the expiration date; whichever comes first. Opened and refrigerated (+2°C to +8°C), reagents are stable for up to 12 weeks or until the expiration date; whichever comes first. Unopened reagents stored refrigerated (+2°C to +8°C) are stable up to the expiration date.
Note: Allow reagents, calibrators, QC and patient samples to reach to ambient temperature (+15°C to +30°C) for ~45 min. prior to loading on the Cobas e601 module.

Calibrator information must be updated in the system before use when a new lot number of calibrator is used for calibration. Review calibrator information after update. Check calibrator status in the system before running QC and patient samples. Calibration status can be checked by going to the **"Calibration**" tab; next go to **"Calibration Status"** tab. Any test highlighted gray requires calibration. Calibration status can also be checked in the system overview page by selecting **"Calibration and QC Select"** and if calibration update is required **"Calibration and QC Select"** will be yellow in the status window. Touch under calibration on "**Recommended"** and under QC on **"Routine QC"** to confirm if calibration and/or QC status is required. Ensure rack position is assigned to non-barcoded calibrators.

b) *Analytical Measurement Range*

Reportable Range: 0.500 – 2000 ng/mL (defined as the lower detection limit and the maximum of the master curve)

Samples with ferritin concentrations above the measuring range can be diluted with Roche **Diluent Universal**. The recommended dilution is 1:50 (either automatically via the rerun function or manually). The maximum dilution for a sample must give a concentration >40 ng/mL.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration. After manual dilution, multiply the result by the dilution factor.

Results less than 15 ng/mL and greater than 400 ng/mL will be repeated to confirm the results.

Calibration verification

Calibration verification is **not required by the manufacturer**.

According to the updated CLIA regulations from 2003 (see also statement of the Joint Commission on Accreditation of Healthcare Organizations), the requirement for calibration verification is met if the test system's calibration procedure includes three or more levels of calibration materials, **and** includes a low, mid, and high value, **and** is performed at least once every six months. All these conditions are met with the calibration procedure of this assay, and therefore no additional calibration verification is required by CLIA.

Perform calibration verification whenever any of the following occur:

- All of the reagents used for a test procedure are changed to new lot numbers, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes.
- There is major preventative maintenance or replacement of critical parts that may influence the test's performance. This includes when the laboratory sends a test system to the manufacturer for repairs. The laboratory must check the calibration of a repaired test system before resuming patient testing and reporting results.
- Control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.
- The laboratory has determined that the test system's reportable range for patient test results should be checked more frequently.

We participate in the **College of American Pathologists (CAP) LN5 Ligand calibration verification/linearity PT challenge twice a year.** The Roche assay has a peer group, and this challenge is evaluated for both linearity and calibration verification. We also participate in the **CAP K Ligand Assay-General survey three times per year.**

Analysis of international standard reference material (SRM) at least twice a year can be used to satisfy calibration verification requirements if the SRM material covers the reportable range of the assay and has at least 3 levels. Reference materials are stored deep frozen (-50°C to -90°C).

The **NIBSC Code 94/572 3rd International Standard for Ferritin, Recombinant (NIBSC 94-572 Serum ferritin recombinant.pdf)** is available for calibration verification. While it is only a one-level material, the concentration is ~3x higher than the upper end of the calibration curve and can therefore be diluted to cover the reportable range of the assay.

- Assigned content of 6.3 micrograms/AMPOULE.
- Reconstitute standard with 1 mL H2O to a concentration of 6300 ng/mL; Run this standard using the instrument auto dilution function (Decreased 1:50).
- Make the following dilutions using the STOCK STANDARD:
  - (A = 1:4) 1575 ng/mL = 250 µL of 6300 ng/mL + 750 µL of Roche diluent universal

- (B = 1:10) 630 ng/mL = 100 µL of 6300 ng/mL + 900 µL of Roche diluent universal
- (C = 1:50) 126 ng/mL = 50 µL of 6300 ng/mL + 2450 µL of Roche diluent universal
- (D = 1:200) 31.5 ng/mL = 50 µL of Soln. B + 950 µL of Roche diluent universal
- (E= 1:1000) 6.3 ng/mL = 50 µL of Soln. D + 200 µL of Roche diluent universal

- At least two replicates of each dilution are run as unknowns against working standards. The measured concentrations should be within ±15% of the expected concentrations (same requirement is used by CAP). A linear regression is generated from expected concentrations as X and measured concentrations as Y. The correlation coefficient should be 0.95 or higher and the slope should be 1.00 $\pm$ 0.10.
- Remaining 6300 ng/mL solution can be used to make additional dilutions. Store these solutions deep frozen at -70^0 C (-50°C to -90°C) for future use.

Ferritin kits were validated for use on the Roche e601 in August 2016.

B. Instrumentation:

JA-4046-I-01-Instrument Maintenance & Function Checks

Ensure the analyzer is in shutdown status or in an appropriate maintenance mode prior to maintenance actions. For most routine maintenance actions (e.g., daily, weekly, and bi-weekly) the e601 module needs to be in standby mode.

Daily Maintenance: Daily maintenance includes cleaning the reagent probe, sample probe, sipper probes and Pre-wash sippers to remove residual solution and precipitation. After cleaning the probes, discharge and operation is checked. When cleaning, take care not to bend or damage the probes or sippers. Impurities on the sample probe may cause problems and affect results. Materials required for daily maintenance of **e601** module are lint-free gauze squares, Alcohol (e.g., isopropyl alcohol or ethanol), deionized water (diH_2O) & paper towels.

For probe maintenance choose > **Utility** > **Maintenance** & choose **(29) next select > Manual Cleaning** from **Maintenance** items list to display the **Manual Cleaning** window > Select **e601** module (*deselect all other modules and units*) >choose **Execute** to complete the task. The probes on the selected module(s) move to their cleaning positions. When instrument movement stops, the manual cleaning can be performed.
For cleaning probes first touch a screw on the rack sampler unit to ground any static charge. Open the top cover; to ensure no alcohol drops into the module, place a paper towel underneath the probe. Clean the probes and sippers using the following procedures:

> ***Note:*** *Do not bend or damage the lower end of the probes during cleaning. Move the arm gently with no up and down movements. Use new lint-free gauze square for each probe to prevent cross contamination. Don't bend the electrode for LLD inside the reservoir. If they are bent, contact the Roche service representative.*

Sample probe: Wipe the probe from top to bottom in a downward motion with lint-free gauze square soaked in diH_2O. If the probe still appears dirty, wipe the outer surface with lint-free gauze square soaked in alcohol, then immediately with diH_2O.
Reagent probe, Sipper probes (for measuring cells 1 & 2) and Pre-wash sipper and dispenser probe: Wipe each of the probes from top to bottom in a downward motion with lint-free gauze square soaked in alcohol, followed by lint-free gauze square moistened with diH_2O.
After cleaning remove the paper towels from the module, close the top cover, lock it and, choose **Stop** (Global button), choose **Yes** to stop maintenance after confirmation.
After maintenance is completed return the probes to standby positions: choose **Utility** >**Maintenance >** choose **(1) next select > Reset** from the **Maintenance** items list. In **Reset** window > select "**e601 module**" (*deselecting other modules and units*) >choose **Execute** to complete the task. The probes on "**e601 module** return to their standby positions. Print the cell blank measurement results and add to calibration binder.

Weekly Maintenance: Weekly maintenance includes cleaning of ProCell/CleanCell nozzles and replacing reservoirs**.** As ProCell dries, crystals are formed. To prevent problems, the ProCell/CleanCell (PC/CC) filling nozzles, and electrodes are cleaned, and the reservoirs are replaced regularly. Materials required are cotton tipped applicator sticks, diH_2O, and 2 ProCell/CleanCell reservoirs. Prior to maintenance the instrument is put in standby mode and maintenance (divided in 5 procedures) is performed as described here:

Procedure 1: To empty ProCell/CleanCell reservoirs choose **Utilit**y >**Maintenance** >select from maintenance list (**33**) to display empty PC/CC select > choose "**Execute"** the Empty PC/CC reservoir step.
Procedure 2: To clean nozzles and electrodes open the top cover, move the arm gently; ensure lower ends of sipper probes don't bend during cleaning. Ensure the electrode for LLD inside the reservoir doesn't bend (*if LLD is bent contact service representative)*. Manually move the sipper probes over the incubator; using the black handle

gently pull up the unit containing PC/CC supplier nozzles and the electrodes to the **"stop/hold position"**. Using cotton tipped applicator sticks soaked in diH_2O clean the PC/CC reservoir filing nozzles (**B**) and electrodes (**C**).
Procedure 3: To replace ProCell/CleanCell reservoirs (**A**) carefully remove PC/CC reservoir; ensure electrodes don't bend & dispose of the PC/CC reservoirs in appropriate waste container. Using a cotton tipped applicator stick soaked in diH_2O wipe and clean the inside of the reservoir positions. Replace PC/CC reservoirs in reservoir positions and push the sipper nozzle unit back into place. Close and lock the top cover. Choose **Stop** (on Global button) after cleaning is complete > choose **Yes** to stop maintenance after confirmation.
Procedure 4: To perform Reagent Prime >choose **Utility** > **Maintenance** > choose **(8) for Reagent Prime** from **Maintenance** items list >Choose **Select to** display the **Reagent Prime** window > **Select** the **e601 module** > enter **Prime Cycles** (1) for the **Reagent** & choose **Execute** to start reagent priming. Wait until the reagent prime is completed and the instrument enters standby mode.
Procedure 5: To perform finalization **>** choose **Utility** > **Maintenance** >choose **(32) Finalization** in the **Maintenance** items list >choose **Select** to display the **Finalization** window & select the e601 module; the channel 1+2 is activated >choose **Execute** & start finalization; complete the procedure & ensure the system returns to standby.
Additional weekly cleaning of other components on **e601 module** includes cleaning of "mixing and separations stations" pre-wash areas; incubator, and assay cup vortex mixer using a lint-free gauze soaked or cotton tipped applicator sticks soaked in diH_2O. Microbead mixer (paddle shaft & propeller plates) is cleaned with lint-free gauze soaked in alcohol followed by diH_2O; rinse stations are cleaned with (1) alcohol-soaked lint-free gauze pads (2) 2% ECO-Tergent followed by cleaning with 50 mL of diH_2O.

Bi-Weekly Maintenance: Contamination in the sipper system could cause measurement problems. To maintain the integrity of the measuring cell cleaning of liquid flow path using **"SysClean"** solution is performed every 2-weeks or after 2500 to 3000 determinations per channel whichever comes first. To perform the cleaning choose **Utility** > **Maintenance** & choose **(27) Liquid Flow Cleaning** from **Maintenance** items list > **Select** to display the **Liquid Flow Path** window. Fill in the SysClean Adaptor M with **SysClean solution. S**elect the e601 module & select channel 1+2 > choose **Execute to** start finalization; complete the procedure & ensure the system returns to standby. Remove the SysClean Adaptor **M**; dispose of any remaining **SysClean solutions** and rinse Adaptor thoroughly with diH_2O.

3-Month Maintenance: Pinch valve tubing in e601 module is replaced (or after 25,000 tests per cell) as tubing wears out in the course of time. Choose maintenance item (**26**) MC Exchange before removing the tubes to avoid fluid drippings on the valves. While instrument is either shutdown or put in standby mode locate pinch valve tubing and carefully remove (pull) all 4 pinch valve tubing from the fittings; dispose of used tubing in appropriate waste container; using a dry gauze pad absorb any liquid draining either from the acrylic block or from the tubing. Carefully insert a new pinch valve tubing through the pinch valve by sliding the ends of the tubing over each fitting.

After pinch valve tubing is replaced perform (**24**) Sipper Air Purge & (**25**) MC Preparation. To perform Sipper Air Purge, start up the instrument, complete initialization; choose **Utility** > **Maintenance** >Select **(24) Sipper Air Purge** from the **Maintenance** Items list >choose **Select >** display **Sipper Air Purge** window; enter **Cycles (10)**>choose **Execute** to initiate the Sipper Air Purge. Ensure that no air remains in the syringe for air, and no leaks are visible around its fittings. Check that enough water is discharged from the sipper probe.
To perform MC Preparation, choose **Utility** > **Maintenance** >Select **(25) MC Preparation** window > Select **Channel >** under Channel Select **Ch1, 2 >** enter 10 in the **Cycles** box (**10**) >choose **Execute** to initiate the maintenance. Ensure there are no leaks at the fittings and on the tubing while the system is performing the maintenance function.

Notes: *Every six months preventive maintenance of the entire Instrument unit is done by Roche service engineer as part of the service agreement. In addition to routine preventive maintenance service agreement includes major repairs or parts replacement and services for these issues are received as needed.*

C. Data Review

JA-4046-DR-01-Computerization & Data System Management

1. **Sample Identification**

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 sample collectors.

2. **Data Collection & Data Back-up**

Roche Cobas® 6000 result files are collected and stored using the software on the Roche Cobas® 6000 workstation. After the data from each run is carefully reviewed, the result files containing patient data as well as QC data are transferred to the NBB Instrument drive on the network via a CDC encrypted USB drive.

3. **Data Import**

The data files are imported from the NBB Instrument drive into the STARLIMS database for QC and statistical evaluation.

4. **STARLIMS Data Review**

Level I – Analyst

- Double click the STARLIMS icon on desktop
- Under 'Run-based Tasks', select 'Pending Runs Assigned to My Labs'
- Choose 'Show Pending Tests' and select test from drop down menu
- Click on 'Add' and select the Instrument
- Run# and Equipment ID will be populated
- [0] Run Instrument Macro – select the excel result file to run macro for STARLIMS import
- [1] Upload Instrument File – import the post-macro result file to STARLIMS
- [2] Mark Null Results – click this button which replaces the null with "|" (a pipe tab)
- [3] Evaluate Sample QC – check the sample QC flags according to the defined criteria
- [4] Evaluate Run QC – evaluate bench QC via the DLS SAS Multi-Rule System QC program to determine QC pass/fail
- [5] Set Run QC Statuses – *set analytes pass/fail based on SAS out-of-control assessment*
- [6] Attach SAS QC file – upload both the SAS input file (.csv) and output file (.pdf)
- Enter run bench QC (SAS) information in Run Comments column
- Click on 'Manage Attachments' and upload the data review checklist for the run
- *Click 'Finish Results' located under the test workflow steps and notify Project Lead*

Level II – Project Lead

- Double click the STARLIMS icon on desktop
- Under 'Run-based Tasks', select 'Run Approval'
- Choose 'Show Pending Tests' and select test from drop down menu
- Review analyst data review checklist and Sample QC evaluation
- Review and confirm Run QC evaluation
- Assess blind QC results - click on 'Blind QC Results Only' tab, 'Assess Blind QC', 'Final Result' and 'Use Default Characterization Sets', 'Proceed to Next Step', 'OK'
- Print blind QC report - click on 'BQC Reports', 'All data displayed in the **data-grid'**, 'A paper-based report from template', 'NBB Blind QC Report v2', 'OK', 'Proceed to the SSRS Report', Save PDF

- Enter bench QC (SAS) and blind QC evaluation status in the Run Comments column
- *Set results final - in 'All Results (S)' tab, click on 'Set Final' Wizard, select 'Process all samples displayed in the '**data-grid'** and 'Run the Set Final Wizard' and click 'Proceed'*
- *Choose Set final criteria - check 'Required Sample QC Passed' and 'Required Run QC Passed'; check 'Pass' and 'Warn' for 'Allowable Results Statuses for Set Final'; choose date range to cover runs that may include the previous analysis of these samples; click 'Proceed'*
- *Resolve samples with retest results and set final*
- Submit sample IDs and repeat instructions to the analyst to schedlue the repeats
- Click on 'Manage Attachments' and upload the blind QC report and Team lead data review checklist for the run
- *In Run Approval tab click Release Run and notify QA Officer (for level III review)*

General Supervisor (Lab Chief)

- Lab chief will conduct random "spot checks" to verify proper handling of lab results
- Discuss with Team Lead or QA Officer course of action on difficult questions
- Results will be set reportable, released, and reported by QA Officer.

JA-4046-DR-02-Out-of-Control Corrective Action

1. QC performance is evaluated by SAS – run comments updated appropriately (pass/fail)
2. SAS QC failure is investigated, and appropriate corrective action measures as indicated are applied.
    - Verify that the proper QC was used.
    - If QCs are failing, Check flags to see if there was problem during sample preparation/analysis.
        - If a QC failed due to sample preparation/analysis issue, apply the appropriate code, flag this QC as rejected & fail (update comment section); exclude failed QC and re-run SAS to check if the remaining QC pass.
        - If run passes notify team lead/supervisor for approval.
    - If the QC failure is true (potential statistical issue); apply code 61 and repeat samples in next run.
    - Enter appropriate comments in STARLIMS database (User fields 1&2).
    - In case QCs failed due to changes in calibrator lot, instrument issues etc. troubleshoot, recalibrate, and re-evaluate otherwise login for service.

JA-4046-DR-03-STARLIMS Data Review Flowchart