Laboratory Procedure Manual

Analyte: **Ferritin**

Matrix: **Serum**

Method: **Bio-Rad Laboratories' QuantImune Ferritin IRMA Kit**

as performed by: *Inorganic Toxicology and Nutrition Branch*
Division of Laboratory Sciences
National Center for Environmental Health

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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 NHANES 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label (and SI units)
lab06	LBXFER	Ferritin (ng/mL)
lab06	LBDFERSI	Ferritin (µg/L)

1. Summary of Test Principle and Clinical Relevance

Ferritin, like hemoglobin, is a major iron storage protein. Isoferritin moieties have been identified for liver and spleen (L isoferritin) and for heart and kidney (H isoferritin). Circulating plasma ferritin is most like the L isoferritin. A serum ferritin assay provides a much more sensitive indicator of iron body stores than a traditional serum iron assay. Serum ferritin levels increase as a result of iron overload, aging, infection, inflammation, liver disease, juvenile rheumatoid arthritis, leukemia, and Hodgkin's disease; and they decrease as a result of iron deficiency.

Ferritin is measured by using the Bio-Rad Laboratories' "QuantImune Ferritin IRMA" kit (1), which is a single-incubation two-site immunoradiometric assay (IRMA) based on the general principles of assays as described by Addison et al. (2) and Miles (3) and modified by Jeong et al. (4). In this IRMA, which measures the most basic isoferritin, the highly purified ^{125}I-labeled antibody to ferritin is the tracer, and the ferritin antibodies are immobilized on polyacrylamide beads as the solid phase. Serum or ferritin standards (made from human liver) are mixed with the combined tracer/solid-phase antibody reagent, and the mixture is incubated. During incubation, both the immobilized and the ^{125}I-labeled antibodies bind to the ferritin antigen in the serum or standards, thus creating a "sandwich." After incubation, the beads are diluted with saline, centrifuged, and decanted. The level of ^{125}I-labeled ferritin found in the pellets is measured by using a gamma counter. There is a direct relationship between the radioactive levels of the pellets and the amount of endogenous ferritin in the serum or standards, rather than the inverse relationship measured by most radioimmunoassays (RIAs).

2. Safety Precautions

The ferritin assay employs ^{125}I as a tracer, and all necessary radiation safety considerations for isotope management and disposal must be observed according to the guidelines of the CDC Radiation Safety Manual. Any laboratory using radioimmunoassay (RIA) kits must hold a current NRC Certificate of Registration and conform to all of the storage, handling and disposal requirements. In addition, all personnel must successfully complete the CDC training course Radiation Safety in the Laboratory or demonstrate having received equivalent instruction. Consider all serum specimens for analysis to be potentially positive for infectious agents including HIV and hepatitis B viruses. Observe Universal Precautions; wear safety glasses, protective gloves, and lab coat during all steps of this method because of both infectious and radioactive contamination hazards. (We recommend the hepatitis B vaccine series for all analysts working with intact blood and serum sample materials.) Place all plastic and glassware that contacts serum other than that which is contaminated by the radioactive tracer in a plastic autoclave bag for disposal. Dispose of all radioactive waste and contaminated material according to radiation safety guidelines.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDS) for these chemicals are readily accessible as hard copies in the lab. If needed, MSDS for other chemicals can be viewed at <http://www.ilpi.com/msds/index.html> or at <http://intranet.cdc.gov/ohs>.

3. Computerization; Data System Management

a. Calculation of serum ferritin (Fer) values is accomplished with the software on the Packard Cobra gamma counter. Ferritin results are manually entered into a Microsoft Excel result file spreadsheet. After a run is complete and any additional corrections by the analyst are made, the Excel result file (containing the patient data as well as the QC data) is electronically transferred to the appropriate analyte-specific subfolder in Q:/ITN/Nutrition Lab/Import into Access on the NCEH/DLS Local Area Network (LAN). The analyst also gives a hardcopy of the result file to the reviewing supervisor. After the reviewing supervisor approves the final values for release by checking off the bench and blind QC values and signing the hardcopy, he/she sends an email to the computer support staff that the data has been released to be imported into the NHANES 1999+ database that is located in Microsoft Access; the computer support staff imports the data into the NHANES 1999+ database by using a macro. Data entry is verified by the computer support staff and the supervisor. Data is transmitted electronically several times weekly to Westat’s ISIS computer system, and transferred from there to NCHS. Abnormal values are confirmed, and codes for missing data are entered by the analyst and are transmitted as part of the

data file to the Westat ISIS computer, and are eventually forwarded to NCHS. Westat also prepares the abnormal report notifications for the NCHS Survey Physician.

b. Files stored on the network or CDC mainframe are automatically backed up nightly by DLS LAN support staff and CDC Data Center staff, respectively. Backup of the daily data containing all raw data files and result files for each run are the responsibility of the analyst. Typically these files are backed up once a week onto a floppy disk or a CD-ROM using a CD writer.

c. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

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

a. No special instructions such as fasting or special diets are required. Diurnal variation is not a major consideration.

b. Specimens for ferritin analysis should be fresh or frozen serum. Serum specimens may be collected by using regular red-top or serum separator Vacutainers.

c. A minimum sample volume of 100 µL is required for the assay; 250 µL will permit repeat analysis as well.

d. Specimens may be stored in glass or plastic vials as long as the vials are tightly sealed to prevent desiccation of the sample.

e. Because ferritin is very stable, serum may be frozen at –20°C to –70°C for years before analysis. Several freeze-thaw cycles do not seem to adversely affect the assay.

f. Specimens should generally arrive frozen. Refrigerated samples may be used provided they are brought promptly from the site of collection.

g. Hemolyzed specimens should not be used because red blood cells contain H isoferritin.

h. Specimen handling conditions are outlined in the Policies and Procedures Manual of DLS (copies are available in the Nutritional Laboratory and the electronic copy of this file is located at Q:/ITN/Nutrition Laboratory/CLIA). The protocol discusses collection and transport of specimens and the special equipment required. In general, plasma should be transported and stored at no more than –20°C. Samples thawed and refrozen less than five times are not compromised. If there is more than one analyte of interest in the specimen and it needs to be divided, the appropriate amount of blood or plasma should be transferred into a sterile Nalge cryovial labeled with the participant's ID.

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

Not applicable for this procedure

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

a. Reagent Preparation

(1) Ferritin tracer/immunobeads
This is supplied as a single reagent, ready to use. Mix the contents to resuspend the immunobeads (the solid-phase antibody-coated matrix for the assay) prior to use. If not used in one run, store at 2–8°C until the expiration date. This material contains ^{125}I and should be properly handled with gloves and disposed according to CDC radiation safety guidelines.

(2) 0.9 g/dL NaCl solution
Place 9 g NaCl in a 1-L volumetric flask and dilute to volume with deionized water.

(3) "Lyphochek" levels I, II, III, and anemia control
Rehydrate by adding 5.0 mL deionized water to each vial of levels I-III and 3.0 mL deionized water to the anemia control. (Bio-Rad says that these quality control materials may be stored up to 10 days at 2–8°C. Our usual practice is to rehydrate multiple vials of the same lot of a level, mix them well, aliquot 0.5 mL into 2.0-mL polypropylene vials, and store them at –70°C to provide us with homogeneous long-term quality control pools for our studies. One vial of each level is thawed for use on the day of analysis.)

b. Standards Preparation

Ferritin standards
These materials (0, 5, 10, 25, 100, 250, 1000 and 2500 ng/mL) are supplied in a liquid form, ready to be used. If the entire kit is not used in one run, store the standards at 2–8°C until their stated expiration date. These standards are prepared by Bio-Rad and were matched to the NIBSC/WHO First International Human Liver Ferritin Standard, available from the National Institute of Biological Standards and Controls, London, UK. The NIBSC material is the only internationally recognized source of purified human liver ferritin.

c. Preparation of Quality Control Materials

Three levels of bench quality control materials are used. QC materials may be purchased commercially (Lyphochek controls) or prepared from pooled human serum.
Lyphochek controls are bought in bulk, rehydrated with deionized water, aliquoted into 2-mL Nalge cryovials, and stored at –70°C. Approximate values are 50, 170, and 350 ng/mL. (See Section 6.c.(3) for preparation instructions.)

Two levels (low-normal and high-normal ferritin concentrations) of blind QC pools may be prepared from pooled, filter-sterilized human serum obtained from fasting donors with elevated or decreased ferritin levels. Pool serum in acid-cleaned 20-L glass carboys. Mix well on a magnetic stirrer. Clean-filter the serum through in a sequential manner using filters of the following pore sizes, each preceded by a pre-filter: 3.00-μm, 1.20-μm, 0.80-μm, 0.65-μm, 0.45-μm, 0.30-μm, and 0.22-μm.

Through the use of sterile technique under a laminar-flow hood, dispense the serum in 1-mL aliquots with a Micromedic Digiflex dispenser into 2.0 mL Nalge cryovials. Cap and label the vials with NHANES bar-coded labels that have been specially prepared for the QC pools. Store the pools at ≤ –70°C at the CDC CASPIR Specimen Repository in Lawrenceville where they will be inserted randomly into the NHANES runs. Select 20 vials of each level at random for characterization of the quality control limits and for testing of homogeneity.

d. Other Materials

(1) Bio-Rad Laboratories' "QuantImune Ferritin IRMA" ^{125}I-ferritin assay kit, 1000-test size (Bio-Rad Laboratories).

(2) "Lyphochek" 3-level ferritin quality control materials and "Lyphochek Anemia Control" lyophilized human serum materials (ECS Division, Bio-Rad Laboratories).

(3) Disposable 12- x 75-mm polypropylene tubes (American Scientific Products, McGaw Park, IL).

(4) Sodium chloride (NaCl), ACS certified (Fisher Scientific Co., Fairlawn, NJ).

(5) "FOAMRAC" foam rubber racks for holding tubes for decanting and blotting after centrifugation (Bio-Rad Laboratories, Hercules CA).

(6) Filters, 3.00-, 0.80-, 0.65, 0.45, 0.30, and 0.22-μm pore sizes (Millipore Corp., Bedford, MA).

(7) Combi-tips, 5.0-mL capacity (Brinkmann Instruments).

e. Instrumentation

(1) Packard Cobra gamma counter, model E5005, (Packard Instruments, Downers Grove, IL) or ICN Model 10/600 Plus gamma Counter (ICN Biomedical, Costa Mesa, CA).

(2) Beckman J6-B centrifuge, 222-tube capacity (Beckman Instruments, Inc., Palo Alto, CA), or Beckman TJ-6 centrifuge (Beckman Instruments).

(3) Gilson Pipetman pipettes, 50- and 200-μL size (Rainin Instrument Co., Woburn, MA).

(4) Eppendorf Repipettor (Brinkmann Instruments, Westbury, NY).

(5) Packard Multiprobe Liquid Handling System (Packard Instruments, Downers Grove, IL).

(6) Thermolyne Maximix III (VWR, Marietta, GA).

(7) Brinkmann Dispensette (Brinkmann Instruments).

(8) Micromedic Digiflex Automatic Dispenser/Dilutor, with 2.0-mL dispensing and 200-μL sampling syringes (Titertek Instruments, Inc., Huntsville, AL)

7. Calibration and Calibration Verification Procedures

Ferritin kit calibration standards are prepared by Bio-Rad and are matched to the latest production lot of NIBSC/WHO International Human Liver Ferritin Standard, available from the National Institute of Biological Standards and Controls, London, UK. These standards are run daily. This method results in a linearized 8-point (including zero) standard curve showing a direct relationship between radioactivity levels measured in counts per min (cpm) and the ferritin concentration in the serum sample. Serum results are expressed as nanograms of ferritin per milliliter of serum (ng/mL).

a. Performance Checks for the Assay

- Nonspecific binding: The zero standard is used as the indicator of nonspecific binding. The cpm for the zero standard tube should be <6% of the cpm of the total counts tube.
- Maximum binding: The cpm of 200 μL of the 2500 ng/mL standard tube should be >45% of the cpm of the total counts tube.
- R^2 should be 0.9900 or greater.

In addition, dilutions of the NIBSC ferritin standard # 80/578 [2nd preparation] are run every 6 months to verify system calibration. This standard material consists of 9.7 μg/ampule purified human liver isoferritin, which, when reconstituted with 1.0 mL of water, yields 9.1 μg/mL ferritin. NIBSC standards are diluted to 0.91, 9.1, 91, and 910 ng/mL. For the standard dilutions tested in June 1991, the slope of the regression line of the expected vs. calculated values was 1.018, the y-intercept was 0.8, and the r^2 was 0.9935. This correlation is re-verified semi-annually. A newer lot of the NIBSC material, #94/572, [3rd preparation] has also been obtained. It contains 6.3 μg/ampule, with an expected ferritin concentration of the reconstituted material stated as 6.3 μg/mL.

b. Calibration of Instrument

The Packard Cobra gamma counter is used for data reduction. To ensure the accuracy of test results, operators must do the following:

(1) Daily: Background and efficiency are run simultaneously using Packard Pico calibrator and associated software. Efficiency should be at least 75% for I125 and 80% for Co57. Printout indicates if all performance parameters are within acceptable limits.

(2) Monthly: Perform normalization with Pico calibrators according to procedure outlined in Packard Procedure manual. Printout will indicate if performance parameters are within acceptable limits.

(3) Semiannually: Preventative maintenance through Packard Technical Service Inspection.

c. Instructions for Calibration of Instrument

(1) Load Picocalibrators in positions 2, 6, 10, 14 and 18, with blank tubes in positions 1, 5, 4, 13, 17 for each isotope.

(2) Insert protocol 25 clip into rack if measuring I125, clip 26 if measuring Co57.

(3) Select F2 (SC Commands), F6 (next protocol)

8. Procedure Operating Instructions; Calculations; Interpretation of Results

a. Manual Pipetting

Follow steps 1–4 to prepare reaction tubes for analysis:

1) Label 12- x 75-mm tubes in duplicate for each standard, control, patient sample, and total counts.

2) Add 50 μL of standard, control, and patient sample to their replicate tubes. EXCEPTION: Add 200 μL of the 2500 ng/mL standard to each of 2 tubes to provide a maximum binding tube for the logit/log data reduction calculations.

3) Thoroughly resuspend the tracer/immunobeads and add 200 μL to all tubes including the total counts tubes.

4) Mix by shaking each rack of tubes. Set aside total counts tubes until the run is ready to be counted.

b. Using Packard Multiprobe

Follow steps 1-5 to prepare reaction tubes for analysis:

1) The layout information is located in the software program assigned to Ferritin.

2) Load samples as specified in the layout.

3) "Execute" the protocol for the ferritin procedure. The parameters and values are programmed into the protocol.

4) Add tracer/immunobeads manually because the timing of this step is important and the autodiluter takes too long.

5) Mix by vortexing the entire rack of tubes on the multi-tube vortexer. Set speed to 1400, hold tubes down with a foam rack and switch power on. Hold 5 sec.

c. Procedure Following Completion of Manual or Autodilutor Steps

1) Incubate tubes at room temperature (about 21-30°C) for 30 min.

2) Add 3.0 mL normal saline to all tubes (except the total counts tubes) and centrifuge 10 min at 1500 x g to pack the solids at the bottom of the tubes. Proceed promptly to the next step; mixing after the saline addition is unnecessary.

3) Place the tubes in the FOAMRACs and invert them over a container designated for radioactive waste in order to discard the supernates. (A large plastic funnel or dishpan is useful for collecting the liquid and channeling it into a plastic bottle for proper disposal of the radioactive waste.) Remove the last drops of liquid by blotting the tube rims on plastic-backed absorbent paper.

4) Place the tubes in racks and count for 1 min in the gamma counter. Record the counts.

d. Packard Mutiprobe Format Record

Use format which is programmed into Multiprobe software. See Multiprobe manual for instructions for optional changes.

e. Calculations

Both the Packard Cobra and 10/600 Plus counters have full data reduction capabilities. Calculation of serum ferritin concentration is accomplished in both counters by using logit B/B_o vs $\log_{10}$ concentration, where:

$$\text{logit } (B/B_o) = \text{Ln}((B/B_o)/(1-B/B_o))$$

and B = corrected cpm (blank subtracted) for each tube, and B_o = maximum binding. (If using the ISODATA software on the Apex counter, use the cubic spline option with a linear curve fit.) In the IRMA, the zero standard is used for nonspecific binding (NSB), and the maximum binding has been experimentally determined to be approximately four times the concentration of the 2500 ng/mL standard.

This method results in a linearized 8-point (including zero) standard curve showing a direct relationship between radioactivity levels (measured in cpm) and the ferritin concentration in the serum sample. Serum results are expressed as nanograms of ferritin per milliliter of serum (ng/mL).

f. Special Procedure Notes – CDC Modifications

On the basis of recommendations by CDC, the manufacturer changed the standard materials (calibrators) used in this kit from the versions originally provided and also included maximum binding tubes to permit automated data reduction with the logit-log function. The kit is then used exactly as outlined by the manufacturer.

9. Reportable Range of Results

The maximum range of detection possible with this method is from 0 to 2500 ng ferritin/mL undiluted serum. Although the commonly observed range is from 0 to 500 ng ferritin/mL serum, linearity has been verified over the range of 0 to 1100 ng/mL with available NIBSC standards. In this laboratory, values less than 10 ng/mL are verified by re-assay, and values greater than 2500 ng/mL are verified by re-assay after the serum has been diluted 1:2 with saline. The ISODATA software reports any values lower than the 5 ng/mL lowest standard as "< 5"; other gamma counters or software may actually quantitate the value. This value is entered in the NHANES 1999+ database arbitrarily as "3." The limit of detection was previously statistically determined with dilutions of the NIBSC standard to be 1.10 ng/mL, but current software does not permit quantitation at this level. In smaller, non-NHANES studies, this result is reported as “less than lowest standard.”

10. Quality Control (QC) Procedures

a. Blind Quality Controls

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

b. Bench Quality Controls

Because of reliability and availability, three levels of Bio-Rad Lyphochek controls are currently used as bench quality control materials. Approximate values are 50, 170, and 360 ng/mL. These pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run.

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

1_{3s} Any of the three QC results are outside the 3s limit

2_{2s} Two of the three QC results in the run are outside the 2s limit (same side of mean)

R_{4s} Sequential QC results (either within the run or across runs) are outside the 2s limit on the opposite sides of the mean

10_x Ten sequential QC results (across pools and across runs) are on the same side of the mean

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared “out of control” for that analyte as assessed by internal (bench) QC.
The initial limits are established by analyzing pool material in 20 consecutive runs and then are reevaluated quarterly. When necessary, limits are updated to include more runs.

While a study is in progress, electronic copies of the QC results from each run are stored in the analyte-specific folder on Q:/ITN/Nutrition Lab/Data handling/Import into Access. Electronic copies of the tracking of the QC results over time are stored in the analyte-specific folder on Q:/ITN/Nutrition Lab/Data handling/QC Results in Excel. A hardcopy of the QC results from each run is also kept by the analyst.

Long-term estimates of method precision from calendar years 1991 and 1992 for NHANES 1999+ (about 200 total runs) show total CVs of about 4–6% at 30–400 ng/mL and 9–10% at 5-10 ng/mL.

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

If the system should be declared "out of control," repeat the entire run. If the "out of control" condition still exists, use a new kit and evaluate the autodiluter for pipetting precision and accuracy. Re-assay specimens for that analytical run after the system has been verified to be "in control."

12. Limitations of Method; Interfering Substances and Conditions

Sources of imprecision in the procedure may be intermittently imprecise micropipettors, an outdated tracer, and the so-called "high-dose hook effect" seen only at extremely elevated levels of ferritin (usually among renal transplant patients or those with hepatic disorders). A repeat analysis of elevated specimens at a higher dilution is performed to confirm their true levels of ferritin.

13. Reference Ranges (Normal Values)

The normal range for serum ferritin is about 10-800 ng/mL for the overall population. Values are lower among females than among males and generally lower among children than among adult females. Values

less than 10 ng/mL usually indicate iron deficiency anemia. Elevated values are caused by iron overload, aging, inflammation, malignancies, hepatic disorders, and juvenile rheumatoid arthritis (1, 2, 5-7).

The data from NHANES 1999+ will be used to describe normal ranges for ferritin for the entire U.S. population. Data from NHANES II describe only a certain subset of the population, and that from HHANES described the Hispanic U.S. population.

14. Critical Call Results ("Panic Values")

The collaborating agency with access to patient identifiers or the responsible medical officer is notified by FAX by the supervisor of any ferritin result that is <10 ng/mL, which possibly represents a significant risk for iron deficiency. Copies of Faxes sent concerning abnormal results are kept in a notebook by the supervisor for the duration of the study. For NHANES 1999+, Westat automatically notifies the NCHS survey physician because of several-times weekly electronic transmission of data.

15. Specimen Storage and Handling During Testing

Specimens should remain at room temperature during preparation and testing.

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

There are no acceptable alternative methods for performing this test for NHANES 1999+. In case of system failure, store all specimens at –20°C until the system is functioning.

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

The collaborating agency with access to patient identifiers or the responsible medical officer is notified by FAX by the supervisor of any ferritin result that is <10 ng/ml, which possibly represents a significant risk for iron deficiency. Copies of Faxes sent concerning abnormal results are kept in a notebook by the supervisor for the duration of the study.

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an ASCII text file or Excel file, either through electronic mail or on a diskette.

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

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

The Microsoft Access database is used to keep records and track specimens for NHANES 1999+. If serum ferritin analyses are used for smaller, non-NHANES studies, records are kept on files in Q:\ITN\Nutrition Lab on the DLS LAN.

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

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored in a freezer at –70°C. The specimen ID is read off of the vial by a barcode reader attached to the computer used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the DIF file containing the electronic copy of the results is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping a notebook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies.

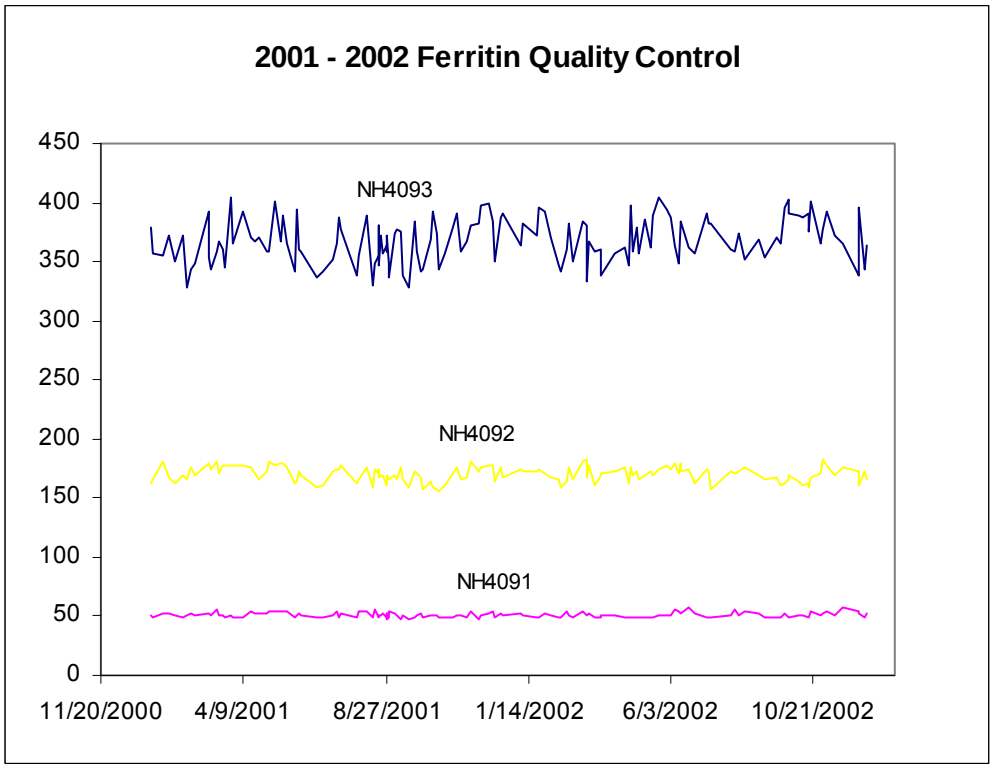
For children less than three years old, serum for the ferritin analysis is obtained from the serum ferritin vial or alternatively from the serum iron/TIBC vial, for all older NHANES participants, serum for ferritin analysis is obtained from the serum ferritin vial or alternatively from the serum folate/vitamin B_{12} vial. Residual serum from either of these vials is retained for serum iron/TIBC repeat analyses.

For studies other than NHANES, samples are stored at –20°C for 1 year after analysis. At this time, the principal investigator is contacted to make a decision concerning storage or disposal of specimens.

19. **Summary Statistics and QC Graphs**

Summary Statistics for Ferritin by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH4041	98	2/9/1999	9/6/2000	24.71	1.40	5.67
NH4042	98	2/9/1999	9/6/2000	132.05	5.06	3.83
NH4043	98	2/9/1999	9/6/2000	420.66	17.50	4.16
NH4091	19	9/22/2000	12/19/2000	52.82	2.10	3.98
NH4092	19	9/22/2000	12/19/2000	176.24	4.14	2.35
NH4093	19	9/22/2000	12/19/2000	373.88	16.43	4.39



REFERENCES

1. Instruction Manual, Bio-Rad QuantImune Ferritin IRMA. Hercules (CA): Bio-Rad Laboratories, 1986.
2. Addison G, Beamish M, Hales C, et al. An immunoradiometric assay for ferritin in the serum of normal patients and patients with iron deficiency and iron overload. J Clin Path 1972; 25:326.
3. Miles L. Measurement of serum ferritin by a 2-site immunoradiometric assay. In: Handbook of Radioimmunoassay (Abraham, G ed.). New York: Marcel Dekker, Inc., 1977: Chapter 4.
4. Jeong H, Blackmore J, Lewin N. Ferritin immunoradiometric assay, U.S. Patent No. 4,244,940
5. Ryan S, Watson L, Tavassoli M, et al. Methods for establishing a working immunoradiometric assay for serum ferritin. Am J Hematol 1978; 4:375.
6. Qvist I, Norden A, Olofsson T. Serum ferritin in the elderly. J Clin Lab Invest 1980; 40:609.
7. Lipschitz D, Cook J, Finch C. A clinical evaluation of serum ferritin as an index of iron stores. N Eng J Med 1974; 290:1213.
8. Gunter EW, Lewis BL, Koncikowski SM. Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES 1999+), 1988-1994. Centers for Disease Control and Prevention, Hyattsville, MD, 1996.

ADDITIONAL SOURCES

Gunter E, Miller D. Laboratory methods used by the Division of Environmental Health Laboratory Sciences, Center for Environmental Health, Centers for Disease Control, for the Hispanic Health and Nutrition Examination Survey (HHANES) 1982_84. Atlanta GA: Centers for Disease Control, 1986. 30-32.

Looker AC, Gunter EW, Cook JD, et al. Comparing serum ferritin values from different population surveys. Vital Health Stat 1991; 2(111): 3-9.

Looker AC, Gunter EW, Johnson CL. Methods to assess iron status in various NHANES Surveys. Nutrition Reviews, 53:9, 246-54, 1995.

Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the U.S. Journal of the American Medical Association, 277:973-976; 1997.

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