Laboratory Procedure Manual

Analyte: **Total Homocysteine (tHcy)**

Matrix: **Plasma**

Method: **Abbott IMX Homocysteine (HCY) Assay**

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

Contact: *Dr. Eric J. Sampson, Director*
Division of Laboratory Sciences

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001–2002 data.

There were two methods used to measure homocysteine in NHANES 2001-2002. Total homocysteine (tHcy) in plasma was measured by the Abbott Homocysteine IMX (HCY) in 2001 and by the Abbott Axsym in 2002.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label (and SI units)
lab06_b	LBXHCY	Homocysteine (µmol/L)

1. Summary of Test Principle and Clinical Relevance

Total homocysteine (tHcy) in plasma is measured by the "Abbott Homocysteine (HCY) assay", a fully automated fluorescence polarization immunoassay (FPIA) from Abbott Diagnostics (1). In brief, dithiothreitol (DTT) reduces homocysteine bound to albumin and to other small molecules, homocystine, and mixed disulfides, to free thiol. S-adenosyl-homocysteine (SAH) hydrolase catalyzes conversion of homocysteine to SAH in the presence of added adenosine. In the subsequent steps, the specific monoclonal antibody and the fluoresceinated SAH analog tracer constitute the FPIA detection system (2). Plasma total homocysteine concentrations are calculated by the Abbott IMx® using a machine-stored calibration curve.

An international round robin performed in 1998 (12) demonstrated that this method is fully equivalent to other most frequently used methods in this field (i.e., HPLC-FD, HPLC-ED, GC/MS). Thus, the Abbott Homocysteine (HCY) assay will be used as primary method for the determination of plasma total homocysteine in NHANES 1999+. The HPLC assay will be used as a reference method and will be performed on a subset of NHANES 1999+ for continuing method comparison and on smaller studies.

Elevated plasma total homocysteine is an independent risk factor for development of a variety of vascular occlusive diseases, including those of the carotid, coronary, and peripheral arteries (3). Increased plasma tHcy can be due to genetic defects or it can be secondary to drugs or certain illnesses. The nutritional influence on mildly elevated homocysteine related to deficiency of folate, vitamin B6 or B12 is of increasing importance. The range of total homocysteine concentration in plasma from "healthy adults" is 5 to 15 umol/L (4). However, the risk for coronary artery disease may significantly increase between 10 and 15 umol/L (5).

2. Safety Precautions

Consider all plasma specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place disposable plastic, glass, and paper (pipet tips, autosampler vials, gloves, etc.) that contact plasma in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

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

3. Computerization; Data System Management

A Statistical evaluation of the runs is accomplished with the Microsoft Excel software ona PC. After a run is completed, plasma total homocysteine concentrations are printed out by the Abbott IMx® instrument. Data are also captured by a RS-232 connection from the instrument to a holding file in Excel. After all the runs of the day and any additional corrections by the analyst are completed, the result file is electronically transferred to a holding file in the NHANES 1999+ Microsoft Access Database on the NCEH/EHLS Local Area Network (LAN). After the reviewing supervisor approves the final values for release, data will be uploaded electronically to the NHANES 1999+ database that is located in Microsoft Access; 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. Westat also prepares the abnormal report notifications for the NCHS Survey Physician.

B. Excel files containing the results are backed up once a week to a floppy disk for storage. The original files are deleted from the hard drive when the analyst is certain that all information has been successfully copied to the diskette. Files stored on the network are automatically backed up nightly to tape by LAN support staff.

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. For best results, a fasting sample should be obtained.

B..Specimens for total homocysteine analysis may be fresh or frozen plasma. Since red blood cells continue to produce and release homocysteine after the blood sample has been obtained, plasma must be separated promptly (6). Freshly-drawn purple-top EDTA Vacutainer tubes collected by standard venipuncture procedures *must be kept on ice water*, and plasma should be harvested within 30 min after drawing.

C. A 200-uL sample of plasma is preferable to allow for repeat analyses; a volume of 50 uL is required for analysis.

D.The appropriate amount of plasma is dispensed into a Nalge cryovial or other plastic screw-capped vial labeled with the participant's ID.

E. Specimens collected in the field are frozen, then shipped on dry ice by overnight mail. Frozen samples are stored at -70°C. Samples are stable for at least 5 years if stored at ≤-20°C (7) and can withstand 5 to 10 freeze/thaw cycles (6).

F. Specimens generally arrive frozen. Refrigerated samples may be used provided they are kept cold and brought promptly (within 2 hours) from the site of collection.

G. Specimens that have been through more than five freeze-thaw cycles, been refrigerated for more than one week, or undergone hemolysis may give inaccurate results.

H. Specimen handling conditions are outlined in the Division protocol for whole blood collection and handling (copies are available in the NHANES laboratory Nutritional Biochemistry Branch Handling Office). 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 labelled with the participant's ID.

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

Not applicable for this procedure

6. Equipment and Instrumentation, Materials, Reagent Preparation, Calibrators (Standards), and Controls

A. Instrumentation

(1) Abbott IMx® system (Abbott Diagnostics, Abbott Park, IL).

(2) Daigger Vortex Genie 2 (VWR, Suwanee, GA).

(3) Multi-tube vortexer (VWR).

(4) Eppendorf micropipet (Brinkmann Instruments Co., Westbury, NY).

B. Materials

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

(1) FPIA carousel.

(2) MEIA carousel.

(3) Blank cells for MEIA carousel.

(4) Sample cartridges for FPIA carousel.

(5) Cuvettes for FPIA carousel.

(6) Probe / electrode.

(7) Fluorometric standards.

(8) Digital thermometer.

(9) FPIA 1 Diluent buffer.

(10)MEIA 2 Diluent buffer.

(11) IMx Homocysteine reagent pack: 4 bottles (ADE/DTT, SAH enzyme, antibody, tracer), 100-test size.

(12) IMx Homocysteine controls: 3 levels (low, medium, high).

(13) IMx Homocysteine calibrators: 6 levels (A through F).

(14) IMx Probe cleaning solution.

This additional reagent is required for cleaning purposes:

(15)Ethanol (Fisher Scientific Co., Fairlawn, NJ.

C. Reagent Preparation

The assay is performed exactly as outlined by the manufacturer. All reagents are supplied by Abbott Diagnostics in liquid form ready to be used. If the entire reagent pack kit is not used in one run, store the kit at 2-8°C until the expiration date is reached. To avoid evaporation of the reagents, the reagent pack kit should not be kept open in the Abbott IMx analyzer when a run is finished.

D. Standards Preparation

Standards (corresponding to 0.0, 2.5, 5.0, 10.0, 20.0, and 50.0 umol/L homocysteine) are supplied by Abbott Diagnostics in liquid form as S-adenosylhomocysteine in buffer, ready to be used. Store the standards at 2-8°C until the expiration date of the kit.

E. Preparation of Quality Control (QC) Materials

(1) Abbott QC pools:
Low (~6 umol/L tHcy), medium (~12 umol/L tHcy), and high (~25 umol/L tHcy) serum based QC pools with specifications concerning the range and the target value are supplied by Abbott Diagnostics, ready to be used, as part of the "Abbott Homocysteine (HCY) assay".

(2) CDC QC pools:
Low, medium, and high plasma based QC pools are prepared and characterized in-house.

To avoid influx of thiols from red blood cells, freshly-drawn purple-top EDTA Vacutainer tubes collected by standard venipuncture procedures *must be kept on ice water*, and plasma should be harvested within 30 min after drawing. This precaution ensures that plasma total homocysteine concentrations are not compromised. All plasma pools are filtered through gauze before being dispensed to remove fibrin. Plasma (250 uL) is aliquoted into 2.0-mL Nalge cryovials, capped, and frozen. The QC pools are stored at -70°C and are stable for at least 3 years.

Means plus range limits for all pools are established by analyzing duplicates for at least 20 consecutive runs.

The low QC pool is prepared by selecting and pooling plasma that contains low levels of homocysteine (~6 umol/L).

The medium QC pool is prepared by selecting and pooling plasma that contains homocysteine mostly at levels representing the critical cut-off point between normal and moderately elevated values (~12 umol/L).

The high QC pool is prepared by selecting and pooling plasma that contains homocysteine mostly at levels representing the critical cut-off point between moderate and intermediate hyperhomocysteinemia (~30 umol/L). Patients with folate/vitamin B12/vitamin B6 deficiency or patients with renal insufficiency have often moderate or intermediate hyperhomocysteinemia. If no plasma with elevated homocysteine concentrations is available, spiking the plasma with known amounts of synthetic L-homocystine is a useful alternative. It is important to use L-homocystine, since SAH hydrolase is sensitive to enantiomeric purity (only the L-form can be used by the enzyme), homocystine is more stable than homocysteine, and using the disulfide accounts also for the reduction step.

7. Calibration and Calibration Verification Procedures

Results of in-house recovery studies showed approximately 102% recovery for various levels of L-homocystine added externally (5, 10, and 20 umol/L tHcy). The accuracy of the "Abbott Homocysteine (HCY) assay" was verified in 1998 with Sigma L-homocystine (20, 40, 100, 200, and 300 umol/L tHcy). The overall slope of the regression line of the expected and calculated values was 0.935, the y-intercept was 0.363, and the r^2 was 0.999. This procedure may be used to reverify the kit accuracy at annual intervals.

During a calibration run (CAL mode), the IMx system assays the calibrators and uses a specific data reduction to generate and store a calibration (Curve 1) from the calibrator assay results. In later performed MODE 2 assays, the IMx uses the calibration to determine the concentration of analyte in patient samples. When a subsequent calibration is performed, the new calibration becomes Curve 1, and the previous Curve 1 becomes Curve 2. The most recent calibration is always stored as Curve 1.

One instance when one would need to use a previously stored calibration (Curve 2) is when one has calibrated with a new reagent lot, but still has some of the previous reagent lot left. To be able to use the previous reagent lot, one may need to use Curve 2, the calibration curve for the previous reagent lot (Section 5a-23 of the IMx Operation Manual).

Calibration is guaranteed by Abbott for a minimum of two weeks. The regular frequency of calibration is monthly. However, the IMx system must be recalibrated if any of the following events occur:

- One or more Abbott QCs or one or more CDC QCs are out of specification.
- The calibration verification is not within 15% of the set value.
- A new lot of reagents is started.
- Any dispense system component is replaced.
- No assays have been performed for two weeks or more.

Calibration is performed in CAL mode (Section 5a-13 to 22 of the IMx Operation Manual) after finishing all the required maintenance (monthly, weekly, and daily). The FPIA carousel is loaded using the following convention: calibrators (in the CAL mode) are *always pipetted in duplicate* into positions 1-12, Abbott controls are pipetted in singlicate into positions 13-15, and the in-house controls are pipetted in singlicate into positions 16-18. **Patient samples must not be run with the calibration.** The calibration has to be accepted and all QC pools have to be in control in order to continue with patient samples. The measured concentrations of the QC pools in this carousel will not be used as QC results for the patient samples that follow in the consecutive carousels.

Calibration verification is performed weekly (unless calibration is performed) by running all calibrators in singlicate as unknowns (MODE 2 assay). The measured concentration of the calibrators must agree within 15% of the set value. **Running patient samples with the calibration verification is optional.** If patient samples are included into the run with the calibration verification, the appropriate number of QC pools has to be included to reach 2 sets of QC pools at the end of the run (day).

NIST reference materials are not yet available for homocysteine assays.

This laboratory participates in the following proficiency testing programs for homocysteine:

Fairview University, Minnesota (7/98 - 10/99) - Tsai/Eckfelt - 5 Specimens twice a year

Aarhus University Hospital, Denmark (1/99 - present) - Christensen/Moeller – 2 Specimens 6 times a year

CAP proficiency testing (4/2000 - present) - 3 Specimens twice a year

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

(1) Allow frozen plasma (patient samples and CDC QCs) to reach ambient temperature.

(2) Perform the required maintenance of the IMx system (monthly, weekly, daily in this order).

B. Preparing the Run

Sequentially run carousels that are separated from one another by less than an hour between ending of one carousel and starting of the next carousel are considered to be part of the same run.

All carousels following the calibration curve and that use the same reagent pack lot will be performed as "MODE 2" assays. In this mode, not all the controls have to be run on each carousel. ***It is required to have a minimum of 2 controls on each carousel, and to rotate through the different QC pool levels during the day to obtain a total of 2 sets of in-house controls and 2 sets of Abbott controls by the end of the day.***

Controls have to be alternated as follows in the consecutive carousels:

9801 – 9805 – 9803 – 9801 – 9805 – 9803 In-house QC pools

Med – High – Low – Med – High – Low Abbott QC pools.

It should be avoided having more than 2 sets of controls per day. If the last carousel of the day contains only repeat patient samples, it is all right to include a third control (level that matches best the majority of the repeat samples). If the measured concentration of this QC pool is in control, the results for the repeat samples will be accepted. The measured concentration of this last QC pool will not be used as QC result for the patient samples.

(1) Place sample cartridges and cuvettes into an FPIA carousel. Be sure that the cuvettes are right-side up or you will have a "boom crash" and the instrument (boom) will have to be recalibrated. Lock the carousel by turning the knob clockwise.

(2) For a calibration run (CAL): Pipette 50 uL of calibrator into the sample well. The first 12 positions will contain A through F calibrator in duplicate. Pipette low, medium, and high Abbott controls in singlicate into the next three positions. Pipette 9801, 9805, and 9803 in-house controls in singlicate into the next three positions.

(3) For a MODE 2 assay: Pipette 50 uL of one CDC controls into the sample well of the first position. Pipette 50 uL of one Abbott control into the sample well of the last position. Pipette 50 uL of patient plasma into positions 2-19.

(4) Ensure that no air bubbles are present in the sample wells. Break a wood applicator into pieces and use them to pop the bubbles.

(5) Place the carousel into the IMx system, making sure the carousel is seated properly.

(6) Gently invert the reagent pack several times. Do not shake the reagent pack, this would create bubbles! Open the reagent pack caps from 1 to 4. Check for large bubbles in the reagent bottles, and pop the bubbles, if necessary.

(7) Place the reagent pack into the reagent block heater (Note: the reagent pack will fit in only one way). Close the IMx door.

C. Initiating a Run

(1) For a calibration run: Select “CAL” from menu on the top right of the instrument. Press “RUN” (above CAL/MODE1/MODE2 menu).

(2) For a MODE 2 assay: Select “MODE2” from menu on the top right of the instrument. Press “RUN” (above CAL/MODE1/MODE2 menu).

D. Creating a Loadlist

(1) Once the instrument has gone to “MULTITASKING”, a loadlist can be created to record sample ID, lot number, and tech ID.

(2) Calibrators are automatically entered by the IMx (in CAL mode). The Abbott controls are entered as follows: press “CONTROL”, then enter “1” for low, “2” for medium, and “3” for high. “AUTO_INC” can be used when samples follow sequentially, e.g. 1000, 1001, 1002, etc.

E. End of Assay

(1) Remove the reagent pack. Close bottles from 4 to 1 and return the reagent pack to the refrigerator.

(2) Make a Xerox copy of the data tape as the data is printed on thermal paper which fades with time.

F. System Maintenance

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

(1) Daily maintenance (Section 9a-1 to 12 of the IMx Operation Manual) should be performed at the start of each 8-hour shift, or more frequently, if necessary. It consists of washing and inspecting the probe electrode assembly, priming the lines of the IMx system and checking for air bubbles and leaks, cleaning the wash station, emptying the waste container and resetting the waste volumen counter, cleaning the probe with TEAH, and performing a FPIA boom check.

(2) Weekly maintenance (Section 9b-1 to 20 of the IMx Operation Manual) consists of cleaning the bar code reader and bar code scanner, cleaning the base air filter, cleaning the carousels and MUP holder, cleaning the dispense system and the waste container, performing a photo check, and a temperature check.

(3) Monthly maintenance (Section 9c-1 to 6 of the IMx Operation Manual) consists of checking the dispense system (dispense check, levels sense check, and sample syringe check) and checking and adjusting the buffer platforms.

G. Special Method Notes

(1) The Abbott IMx® system should always be “ON”.

(2) Turn the system completely off only if it will be idle for more than two weeks, or if the assay module is replaced.

(3) Whenever the system is idle for more than one month, the lines should be primed with water/methanol (80:20).

H. Calculations

All calculations are performed by the IMx system using a machine-stored calibration curve.

I. CDC Modifications

This method is based on the method described by Shipchandler et al. (1) and has been validated and compared to an HPLC assay with internal standardization (8). The method is run exactly as stipulated by the manufacturer; CDC has introduced no modifications.

9. Reportable Range of Results

This method is linear for homocysteine in the range 3-50 umol/L. Samples with results <2 umol/L or >15 umol/L are reanalyzed for confirmation before results are released. Samples with total homocysteine concentrations ≥50 umol/L are diluted 10-fold with PBS or FPIA buffer and reanalyzed. This method has a total coefficient of variation in the range of 3-6%.

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

Since the limits of the Abbott QC pools are fairly wide (to account for instrument variations), they will not be used to decide whether a run (consisting of one carousel of of several carousels) is out of control. This decision will be made based on the results of the CDC QC pools. The Abbott pools will be used to verify proper instrument operation.

CDC bench QC specimens are prepared from three plasma pools, which represent low, intermediate, and high levels of homocysteine in plasma. These pools are prepared in the same manner as patient samples and analyzed 2 times as part of each run.

The results from the pools are checked after each carousel is finished (incomplete set of controls). If the value of one or more bench QC specimens is outside the 99% limits, the run is not continued, and the IMx system is recalibrated. The run is resumed and the previous carousel is repeated.

The results from the pools are checked at the end when the run is complete (2 complete sets of controls). The system is declared “out of control” if any of the following events occur:

For the Means Chart:

- A single run mean for one or more pools falls outside the upper or lower 99% limit.
- The run means of two or more pools fall either both above or both below the lower 95% limit.
- Two successive run means for a single pool fall either both above or both below the lower 95% limit.
- Eight successive run means for a single pool fall either all above or all below the center line, establishing a trend.

For the Range Chart:

- A single within-run range falls above the upper 99% limit.
- The within-run ranges for two or more pools fall above the upper 95% limit.
- Two successive within-run ranges for a single pool fall above the upper 95% limit.
- Eight successive within-run ranges for a single pool fall above the center line.

The initial limits are established by analyzing pool material in 20 consecutive runs and then are reevaluated after 20 additional runs. After that, limits are re-evaluated bi-annually.

While a study is in progress, the supervisor stores hard copies of the QC results from each analysis in a notebook, a copy of which is also kept by the analyst. Electronic copies of the QC analyses are stored on a diskette.

Reanalyze samples with homocysteine concentrations outside of the normal range on a subsequent run. Dilute samples that have a homocysteine concentration greater than 50 umol/L 10-fold prior to reanalysis or run those samples using the IMx dilution assay.

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

Check to make sure that the hardware is functioning properly.
Recalibrate the instrument.
If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

Serum is a less suitable specimen than properly prepared plasma, since erythrocytes still produce and release homocysteine during the blood clotting. Improperly prepared plasma (not separated from the red cell within 30 min) may also be unsuitable. Total homocysteine concentrations may be overestimated in these samples.

Very lipemic specimens may show a discrepancy between the Abbott Axsym® and the HPLC result. They should be measured by the Abbott Homocysteine assay both undiluted and diluted with PBS (1:2 or 1:3), and they should also be measured by HPLC (undiluted and diluted). The diluted sample should be reanalyzed, if results between the undiluted and diluted sample are discrepant.

13. Reference Ranges (Normal Values)

Based on literature data (10, 11), the current proposed normal and elevated ranges for this method are shown below. Preliminary reference ranges for plasma total homocysteine will be established using the data from the first year of NHANES 1999+.

Table 1.
Homocysteine Reference Ranges

	umol/L Total Homocysteine
Normal range	4.6 - 8.1 < 30 years
	4.5 - 7.9 30-59 years, females
	6.3 - 11.2 30-59 years, males
	5.8 - 11.9 > 60 years

Moderate hyperhomocysteinemia	16 - 30
Intermediate hyperhomocysteinemia	31 - 100
Severe hyperhomocysteinemia	> 100

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 homocysteine results that is >15 umol/L, which possibly represents a significant risk for cardiovascular disease. Copies of FAXes sent concerning abnormal results are kept in a notebook by the supervisor for the duration of the study. For NHANES 1999+, since data are transmitted several times weekly to the Westat ISIS computer, Westat automatically notifies the NCHS survey physician.

15. Specimen Storage and Handling During Testing

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

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

If the analytical system fails, we recommend that the specimens be stored at $\leq$-20 °C until the analytical system is restored to functionality. If the results are needed earlier than the system reaches functionality, specimens can be prepared and analyzed by an HPLC method with fluorometric detection (9).

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 homocysteine results that is >15 umol/L, which possibly represents a significant risk for cardiovascular disease. Copies of FAXes sent concerning abnormal results are kept in a notebook by the supervisor for the duration of the study.

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

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

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

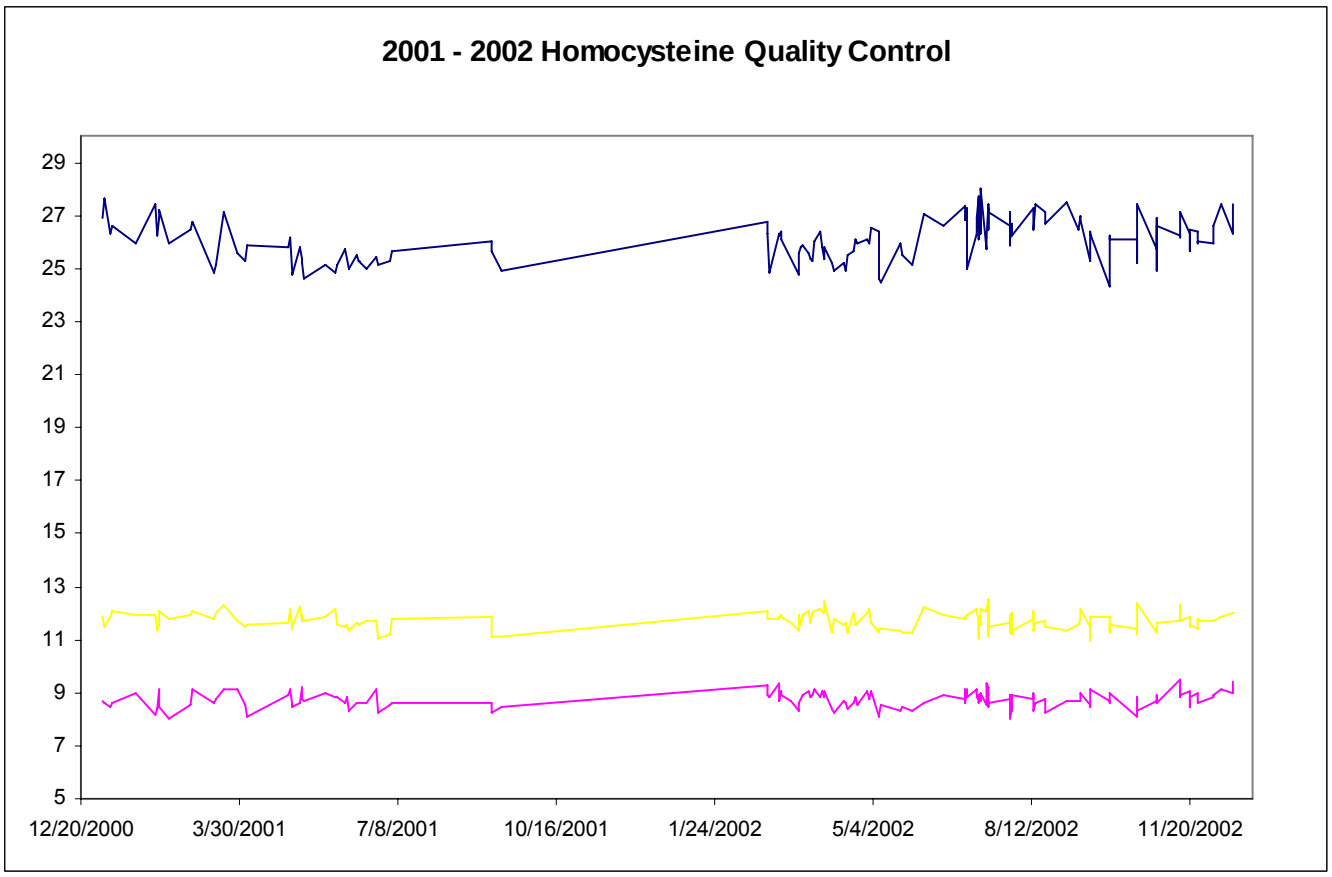
The Microsoft Access database is used to keep records and track specimens for NHANES 1999+. If plasma homocysteine analyses are used for smaller, non-NHANES studies, records are kept on files in L:\link\nbb on the EHLS LAN. We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies may be discarded at the request of the principal investigator, or may be transferred to the CDC CASPIR facility for use by other investigators. Very little residual material will be available after NHANES analyses are completed, and these vials may be routinely autoclaved.

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

19. Summary Statistics and QC Graphs

Summary Statistics for Homocysteine by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
NH2002	152	1/3/2001	12/18/2002	8.73	0.30	3.4
NH2003	152	1/3/2001	12/18/2002	11.72	0.32	2.7
NH2004	152	1/3/2001	12/18/2002	26.11	0.82	3.1



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