



## Laboratory Procedure Manual

*Analyte:* **Total Homocysteine (tHcy)**

*Matrix:* **Plasma**

*Method:* **Abbott 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 2003-2004 data.

A tabular list of the released analytes follows:

<b>Lab Number</b>	<b>Analyte</b>	<b>SAS Label (and SI units)</b>
lab06_c	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, homocysteine, 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 Axsym® using a machine-stored calibration curve. (3-4).

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+. For NHANES 1999–2001, the Abbott Imx was used, starting NHANES 2002, the Abbott AxSym is used. 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  $\mu\text{mol/L}$  (4). However, the risk for coronary artery disease may significantly increase between 10 and 15  $\mu\text{mol/L}$  (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. Calculation of plasma homocysteine values is accomplished with the software on the Abbott instrument. Homocysteine 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 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. 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 500- $\mu$ L sample of plasma is preferable to allow for repeat analyses; a volume of 150  $\mu$ L 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, and then shipped on dry ice by overnight mail. Frozen samples are stored at  $-70^{\circ}\text{C}$ . Samples are stable for at least 5 years if stored at  $20^{\circ}\text{C}$  (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 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  $-20^{\circ}\text{C}$ . 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. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

##### A. 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  $2-8^{\circ}\text{C}$  until the expiration date is reached. To avoid evaporation of the reagents, the reagent pack kit should not be kept open in the Abbott AxSYM analyzer when a run is finished. The Abbott AxSYM uses the same reagents as the Abbott IMx.

## B. Standards Preparation

Standards corresponding to 0.0, 2.5, 5.0, 10.0, 20.0, and 50.0  $\mu\text{mol/L}$  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.

## C. Preparation of Quality Control Materials

### (1) Abbott QC pools:

Low ( $\sim 6 \mu\text{mol/L}$  tHcy), medium ( $\sim 12 \mu\text{mol/L}$  tHcy), and high ( $\sim 25 \mu\text{mol/L}$  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  $\mu\text{L}$ ) is aliquoted into 2.0-mL Nalge cryovials, capped, and frozen. The QC pools are stored at  $-70^\circ\text{C}$  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 ( $\sim 6 \mu\text{mol/L}$ ).

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 ( $\sim 12 \mu\text{mol/L}$ ).

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 ( $\sim 30 \mu\text{mol/L}$ ). 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-homocysteine is a useful alternative. It is important to use L-homocysteine, since SAH hydrolase is sensitive to enantiomeric purity (only the L-form can be used by the enzyme), homocysteine is more stable than homocysteine, and using the disulfide accounts also for the reduction step.

## D. Other Materials

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

- (1) Sample Segments Cartridges
- (2) Reaction Vessels
- (3) Probe / electrode
- (4) Fluorometric standards
- (5) Digital thermometer
- (6) Bulk Solutions 1, 2, 3, 4

- (7) Axsym Homocysteine reagent pack: 4 bottles (ADE/DTT, SAH enzyme, antibody, tracer), 100-test size
  - (8) Homocysteine controls: 3 levels (low, medium, high)
  - (9) Homocysteine calibrators: 6 levels (A through F)
  - (10) Axsym Probe cleaning solution
- This additional reagent is required for cleaning purposes:
- (11) Ethanol (Fisher Scientific Co., Fairlawn, NJ)

#### E. Instrumentation

- (1) Abbott Axsym system (Abbott Diagnostics, Abbott Park, IL).
- (2) Daigger Vortex Genie 2 (VWR, Suwanee, GA).
- (3) Multi-tube vortexer (VWR, Suwanee, GA).
- (4) Eppendorf micropipet (Brinkmann Instruments Co., Westbury, NY).

#### 7. Calibration and Calibration Verification Procedures

Results of in-house recovery studies showed approximately 102% recovery for various levels of L-homocysteine added externally (5, 10, and 20  $\mu\text{mol/L}$  tHcy). The accuracy of the "Abbott Homocysteine (HCY) assay" was verified in 1998 with Sigma L-homocysteine (20, 40, 100, 200, and 300  $\mu\text{mol/L}$  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 Axsym 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 assays, the Axsym uses the calibration to determine the concentration of analyte in patient samples. The Calibration Curve is stored separately for each calibrated lot number of reagent. Once a lot number of reagent is calibrated the instrument recognizes that calibrated lot number of reagent and with performs the assay using the calibration curve for that assay.

Calibration is guaranteed by Abbott for a minimum of two weeks. The regular frequency of calibration is monthly. However, the Axsym 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 are started.
- Any dispense system component is replaced.
- No assays have been performed for two weeks or more.

Calibration is performed by assigning the calibrator A-F to a segment, after finishing all the required maintenance (monthly, weekly, and daily). The Calibrators are loaded onto the segment starting with the lowest calibrator being in the first position of the segment and loading all others subsequently in positions 1-6. When these samples are programmed on the analyzer as Calibrators they will automatically be analyzed in duplicate. Abbott controls and in-house controls are pipetted in singlicate into the positions immediately following the calibrators on the sample segment. 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 during the calibration will not be used as QC results for the patient samples that follow in the consecutive runs.

Calibration verification is performed weekly (unless calibration is performed) by running all calibrators in singlicate as unknowns. 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.

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 AxSYM system (monthly, weekly, daily in this order).

### B. Preparing the Run

One run is defined as a maximum of 6 segments (60 samples) including duplicates of three levels of in-house bench QC pools.

Prepare segments beginning with all three levels of in-house bench QC pools in the first three positions of the segment. Pipette samples in remaining positions of that segment and subsequent segments until you reach the sixth segment. Leave the last four positions on the last segment (sixth segment) for controls. Pipette one level of Abbott QC and all three levels of in-house bench QC (in that order) into the last four positions on the segment.

Each run must contain three levels of in-house bench QC pools on the first segment that starts the run, and on the last segment of the run in the last four positions of the segment, along with one level of Abbott QC.

You may place more than one reagent pack at a time on the analyzer however; avoid using more than one lot number of reagent for a single run.

When performing small runs or confirmation (repeat) runs, you must have all three levels of in-house bench QC in duplicate and all three levels of Abbott QC in singlicate.

- (1) Place sample cups onto a segment and pipette the samples into the sample cup. Be sure to load the samples from left to right starting with the first row of the segment. Be sure to load segments sequentially starting with position 1 on the instrument and the first available segment.

**NOTE:** Be sure to clear order status before beginning each run or at the start of each day.

- (2) For a calibration run: Pipette 150  $\mu$ L of calibrator into the sample cup. The first 6 positions will contain A through F calibrator. Pipette low, medium, and high Abbott controls in singlicate into the next three positions. Pipette in-house controls in singlicate into the next three positions.
- (3) For runs other than Calibration run: Pipette 150  $\mu$ L of all three CDC controls into the first three sample cups on the first segment. Pipette 150  $\mu$ L of patient sample in the next available sample cups on the segment and subsequent segments until you reach the sixth segment. On the sixth segment pipette 150  $\mu$ L of patient plasma into the first 6 positions, in the 7th position pipette one level of Abbott QC and in positions 8–10 pipette low, med and high In-house bench QC.

- (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 segments on the Axsym system starting in position 1 with the first segment and continue placing the segments in numerical and alphabetical order on the instrument, making sure that all segments are 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 carousel, in any position. If you place more than one reagent pack on the carousel, be sure to place the pack with the least volume in a position that precedes the fuller reagent pack.

#### C. Initiating a Run

For all runs, press the green "RUN" button on the to left hand side of the keyboard.

**NOTE:** You must create an order list (program samples) before you can initiate a run. The analyzer will only proceed, if there are samples programmed to be run.

#### D. Creating an Order List

- (1) Be sure to clear all previously programmed samples from the Order Status menu screen.
- (2) In the Order list menu, select either calibrator, control or patient. If selecting calibrators or controls you will then need to select the test. Once the test is selected calibrators will automatically be assigned a segment and cup. You will have to select the level of control and it will automatically be assigned a segment and cup (this applies only for Abbott QC; in-house bench QC is programmed the same as a patient sample).
- (3) When programming patient samples select patient from the Order List menu, then enter or scan the bar-coded patient sample ID number (you may add other comments on this screen such as hemolysis or a name if desired), select the test (tHcy) and touch next.
- (4) Continue with steps 2-3 as needed to match the set up of your segments. When all samples, controls and calibrators have been programmed and loaded, exit the order list screen and go to main menu. From the main menu, press the green RUN key.

**NOTE:** Be sure to load sufficient reagent packs (test) to complete the run. Multiple reagent packs may be loaded on to the analyzer as long as they are the same lot number.

#### E. End of Assay

- (1) Remove the reagent pack. Close bottles from 4 to 1 and return the reagent pack to the refrigerator.
- (2) Once results are complete, proceed to the results menu. Highlight all desired results and touch the print key. Once you have printed all results, touch the release key to release the results into the system database. Once results are released, those segments can be programmed with new samples.

**NOTE:** Be sure to check order status screen before starting a new run. Make sure that order status screen is clear, as well.

#### F. System Maintenance

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

- (1) Daily maintenance (Section 9a-3 to 20 of the AxSYM Operation Manual) should be performed at the start of each 8-hour shift, or more frequently, if necessary. It consists of performing a probe clean procedure, emptying the waste receptacles, checking and replacing the bulk solutions and checking and updating the inventory and resetting all volumes.
- (2) Weekly maintenance (Section 9b- 21 to 66 of the AxSYM Operation Manual) consists of cleaning the outside of the probes, wash stations and dispenser nozzles. Flushing the Pumps and Syringes, cleaning the sample segments and sample cup adapters, cleaning the processing and matrix cell carousels and air filters. Perform FPIA verification.
- (3) Monthly maintenance (Section 9c-67 to 68 of the AxSYM Operation Manual) consists of performing the Tubing Decontamination procedure to be found on page 9-123.

#### G. Special Method Notes

- (1) The Abbott AxSYM system should always be "ON."
- (2) Turn the system completely off only if you will be taking the instrument apart or when indicated by maintenance procedure or error code.

#### H. Calculations

All calculations are performed by the AxSYM 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 2–50  $\mu\text{mol/L}$ . Samples with results  $<2 \mu\text{mol/L}$  or  $>15 \mu\text{mol/L}$  are reanalyzed for confirmation before results are released. Samples with total homocysteine concentrations  $50 \mu\text{mol/L}$  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

Bench QC specimens are prepared from three plasma pools, which represent low, intermediate, and high levels of MMA in plasma. 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.

#### 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 in Table 1 (APPENDIX).

#### 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  $>15 \mu\text{mol/L}$ , 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  $-20^{\circ}\text{C}$  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  $>15\ \mu\text{mol/L}$ , 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 or serum Methylmalonic acid analyses are used for smaller, non-NHANES studies, records are kept on files in Q:\ITN\Nutrition Lab on the DLS LAN.

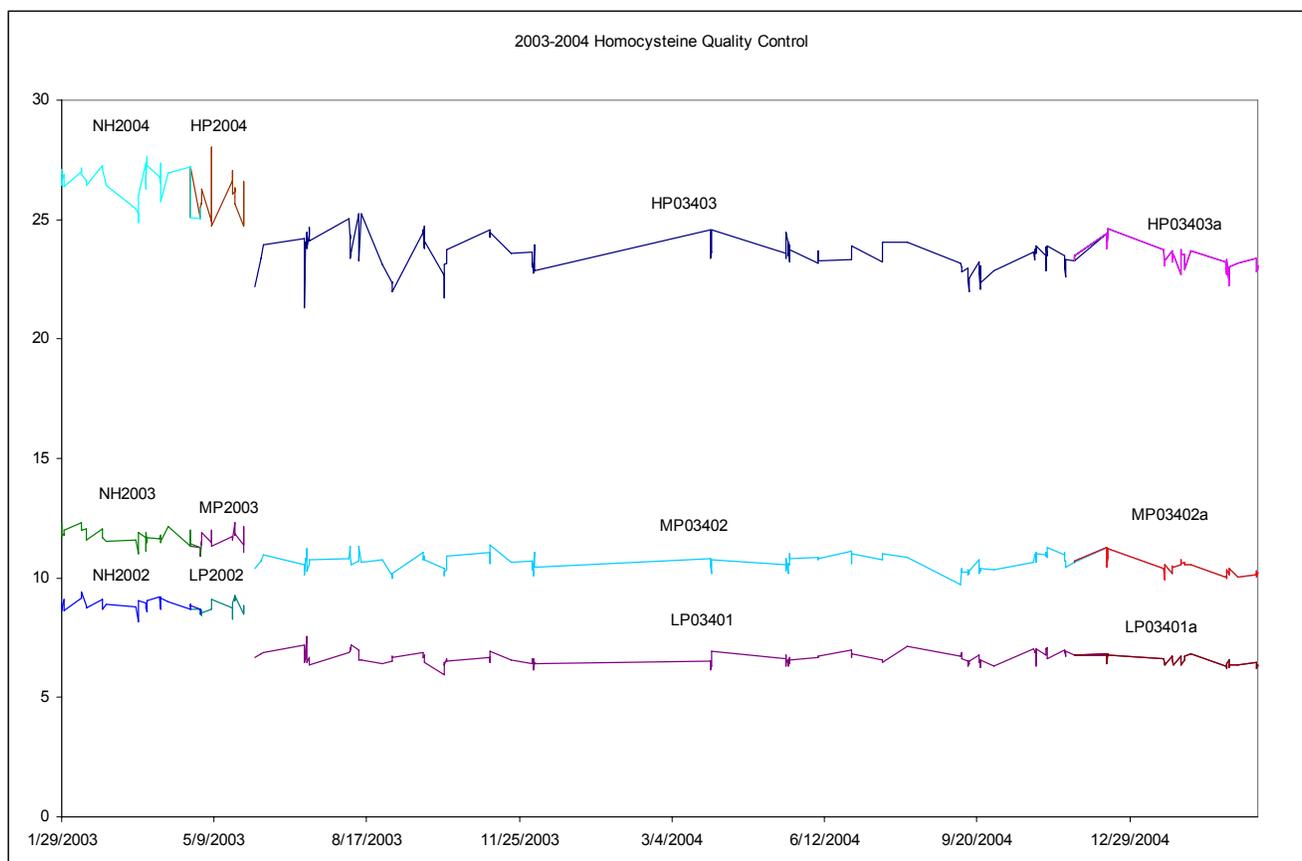
We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum 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  $-70^{\circ}\text{C}$ . 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	34	1/29/2003	4/30/2003	8.874	0.253	2.9
NH2003	34	1/29/2003	4/30/2003	11.704	0.361	3.1
NH2004	34	1/29/2003	4/30/2003	26.528	0.747	2.8
LP2002	20	4/23/2003	5/28/2003	8.761	0.271	3.1
MP2003	20	4/23/2003	5/28/2003	11.599	0.363	3.1
HP2004	20	4/23/2003	5/28/2003	25.966	0.953	3.7
LP03401	106	6/5/2003	12/14/2004	6.664	0.259	3.9
MP03402	106	6/5/2003	12/14/2004	10.656	0.337	3.2
HP03403	106	6/5/2003	12/14/2004	23.509	0.759	3.2
LP03401a	31	11/23/2004	3/23/2005	6.506	0.201	3.1
MP03402a	31	11/23/2004	3/23/2005	10.416	0.329	3.2
HP03403a	31	11/23/2004	3/23/2005	23.326	0.542	2.3



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Appendix

Table 1.  
Homocysteine Reference Ranges

	µmol/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