Laboratory Procedure Manual

Analyte: **Total homocysteine (tHcy)**

Matrix: **Plasma**

Method: **"Abbott Homocysteine (HCY) assay"**

Method No:

Revised:

as performed by:

Inorganic Toxicology and Nutrition Branch
Division of Laboratory Sciences
National Center for Environmental Health

contact:

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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.

Procedure Change Log

Procedure: Total Homocysteine (tHcy) - Abbott DLS Method Code: _____

Date	Changes Made	By	Reviewed By (Initials)	Date Reviewed
Sept 2001	Made changes with regard to how data are reported from analyst to supervisor and how data are imported into Access database.			
Sept 2001	Introduced new QC rules.			
April 2002	Started using Abbott AxSym analyzer instead of IMx when NHANES 2002 was started (same kit).			
July 2005	P.2-4 - Changed write-up for instrument import of data into Access (started March 2005) to more clearly explain steps involved.	Irene Williams		
July 2006	P.8 - Added CAP calibration verification survey.	Christine Pfeiffer		
July 2006	P.9 - It is not required to run more than one level of Abbott QC pools in small repeat runs.	Christine Pfeiffer		
July 2006	P.7 - Added information on newly available SRM material for serum homocysteine.	Christine Pfeiffer		
May 2007	Put references in order and added Pernet reference (specific for AxSYM).	Christine Pfeiffer		

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label (and SI units)
HCY_D	LBXHCY	Homocysteine (µmol/L)

Total Homocysteine in Plasma
NHANES 2005-2006

1. Summary of Test Principle and Clinical Relevance

Total homocysteine (tHcy) in plasma is measured by the "Abbott Homocysteine (HCY) assay" (1), a fully automated fluorescence polarization immunoassay (FPIA) from Abbott Diagnostics performed on the AxSYM® platform (2). 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 (3). Plasma total homocysteine concentrations are calculated by the Abbott AxSYM® using a machine-stored calibration curve.

An international round robin performed in 1998 (4) demonstrated that the Abbott 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® analyzer was used, starting NHANES 2002, the Abbott AxSYM® analyzer is used. The IMx® and the AxSYM® platforms are both using the same reagent kit, but the AxSYM® is a newer fully-automated analyzer that can measure multiple analytes during one run. Pernet et al. showed that the two platforms agree well (2). An in-house 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 (5, 6).

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 (7). 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 5-15 µmol/L (8). However, the risk for coronary artery disease may significantly increase between 10 and 15 µmol/L (9).

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 HCY values are accomplished with the software on the Abbott AxSym instrument. Generated data is copied onto a floppy disk and archived to C: as an ASCII file. Transmission of data from A: to the Microsoft Access Network Database is described below:

Step 1 – Analyst – Import data file into ACCESS:

-Double click the ACCESS icon on desktop, password entry required

-[Add Sample Results to Database] (under Batch & X-Batch)

-[Import Instrument Data File] - Enter information (instrument, assay, date, time, analyst, study)

-[Import] – In "select data file" window, choose A: and import file number assigned for the date/run number. Check that sample ID's are recognized

-[Transfer]

Step 2 – Analyst – Review run in ACCESS:

-[Run Review] (under Batch & X-Batch) – Select assay

-[Show runs] – Cursor to desired run, enter sample set name and comments

-[QC Results] – Review QC results for transmission errors and whether they pass the 2S limits

-[Print Report] [Back]

-[Sample Results] – Review patient results to assure proper information transmission, enter appropriate comment codes on flagged samples

-[Set Final] results that are ready to be reported

-[Set Reviewed]

-[Print Report] [Back]

Step 3 – Analyst – Send email and run folder to QA Officer:

An e-mail is sent to the QA Officer including the following run information: Analysis date, Instrument, Study, Groups, File name, Batch ID, Run #, and QC Status. Noteworthy comments are included in the email. All printouts including raw data are submitted in a run folder to the QA Officer who reviews the Bench QC data via the ACCESS database as described below.

Step 4 – QA Officer – Review Bench QC via ACCESS:

-Double click the ACCESS icon on desktop, password entry required

-[Export QC to SAS] (under Batch & X-Batch) – Select Assay, Date range and Controls

-[Make QC Data Infile] – Save file to I:, appropriate subfolder for archival

-[Run SAS] – SAS will automatically open, [go], review each generated plot, print QC cover page and standard deviation plot, [Back]

-[Run Review] (under Batch & X-Batch) – Select assay

-[Show runs]

-[Sample Results]

-[Set Batch QC] – accept or reject

-[Set Reviewed]

-Forward email from Analyst to Second QA reviewer (for Blind QC review) specifying Bench QC status of the run.

Step 5 – Second QA Reviewer – Review Blind QC and other parameters in ACCESS:

-Double click the ACCESS icon on desktop, password entry required

-[Run Review] (under Batch & X-Batch) – Select assay, then desired run

-[Blind QC Results] – Review whether Blind QC results pass the 2S limits

-[Print Report] [Back]

-Check other parameters if applicable (i.e., background, calibration curve, repeat values, replicates, signal intensity)

-[Set RQC] – accept or reject

-Verify that appropriate comment codes have been applied and that final values have been set correctly

-[Set Reviewed]

-Forward email from QA Officer to Supervisor specifying Blind QC status of the run and other relevant comments.

Step 6 – Supervisor – Approval and Export of Results via ACCESS:

-Double click the ACCESS icon on desktop, password entry required

-[Run Review] (under Batch & X-Batch) – Select assay, then desired run

-Perform final review of Bench and Blind QC status, comment codes, repeat results

-[Set Ready] – Final results will be set ready to be exported

-[Set Reviewed]

-[Export/Report Results] (under Study Functions) – Select study, select analytes/panel, use selected panel

-[Generate Excel Spreadsheet] – Review file on I:\To be transmitted

-[Generate Export Text File and Set Results Exported] – Review file on I:\To be transmitted

-FTP file to Westat

-Send Westat an email that file was transmitted

-After min. of 1 day, move transmittal file from I:\To be transmitted to I:\Transmitted Data\Appropriate Year Folder.

For NHANES, data is transmitted electronically several times weekly to Westat's ISIS computer system and transferred from there to NCHS. Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and

are transmitted as part of the data file 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 (10). 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-µL sample of plasma is preferable to allow for repeat analyses; a volume of 150 µL 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°C. Samples are stable for at least 5 years if stored at ≤-20°C (11) and can withstand 5 to 10 freeze/thaw cycles (10).

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°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°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 µmol/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 (~6 µmol/L tHcy), medium (~12 µmol/L tHcy), and high (~25 µmol/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 µL) 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 µmol/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 µmol/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 µmol/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.

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-homocystine added externally (5, 10, and 20 µmol/L tHcy). The accuracy of the "Abbott Homocysteine (HCY) assay" was verified in 1998 with Sigma L-homocystine (20, 40, 100, 200, and 300 µmol/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.

The AxSYM® Homocysteine assay must be calibrated using a Standard Calibration (6-point) procedure. Once the AxSYM® Homocysteine calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

-A reagent pack with a new lot number is used.
-Controls are out of range.
-A new assay file is installed.
-A new version of an existing assay file is installed.

Calibration is optional, but recommended, if:

-Assay-specific measures of curve fit on the calibration review screen are not within specification.
-A bulk solution with new lot number is used.
-Any dispense system component is replaced.
-Any system maintenance procedure is performed.

Refer to the AxSYM® System Operations Manual, Section 6 for further information on when recalibration or calibration verification may be necessary.

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. **It is not recommended to run patient samples as part of the calibration run.** 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.

On October 6, 2005, the National Institute of Standards & Technology (**NIST**) issued the first standard reference material for homocysteine and folate in frozen human serum (**SRM #1955**) (12). This material has certified concentrations for homocysteine by LC/MS/MS, LC/MS, and GC/MS. It also has orientation values for homocysteine by Abbott AxSYM® and HPLC-FD.

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

CAP LN16 calibration verification (2002 - present) - 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 plus one Abbott QC.

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. The run should include one level of Abbott QC pools.

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 ~160 µL 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 ~160 µL of all three CDC controls into the first three sample cups on the first segment. Pipette ~160 µL 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 ~160 µL 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 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 barcoded 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 Pernet et al. (2) and has been validated and compared to an HPLC assay with internal standardization (6). 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 0.8-50 µmol/L. Samples with results <2 µmol/L or >15 µmol/L are reanalyzed for confirmation before results are released. Samples with total homocysteine concentrations >50 µmol/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, QC results are stored in the ACCESS database. For runs that are not imported into ACCESS (exception, research-type runs), QC results are stored electronically 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 (13, 14), 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 µmol/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°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 (5).

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 µmol/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.

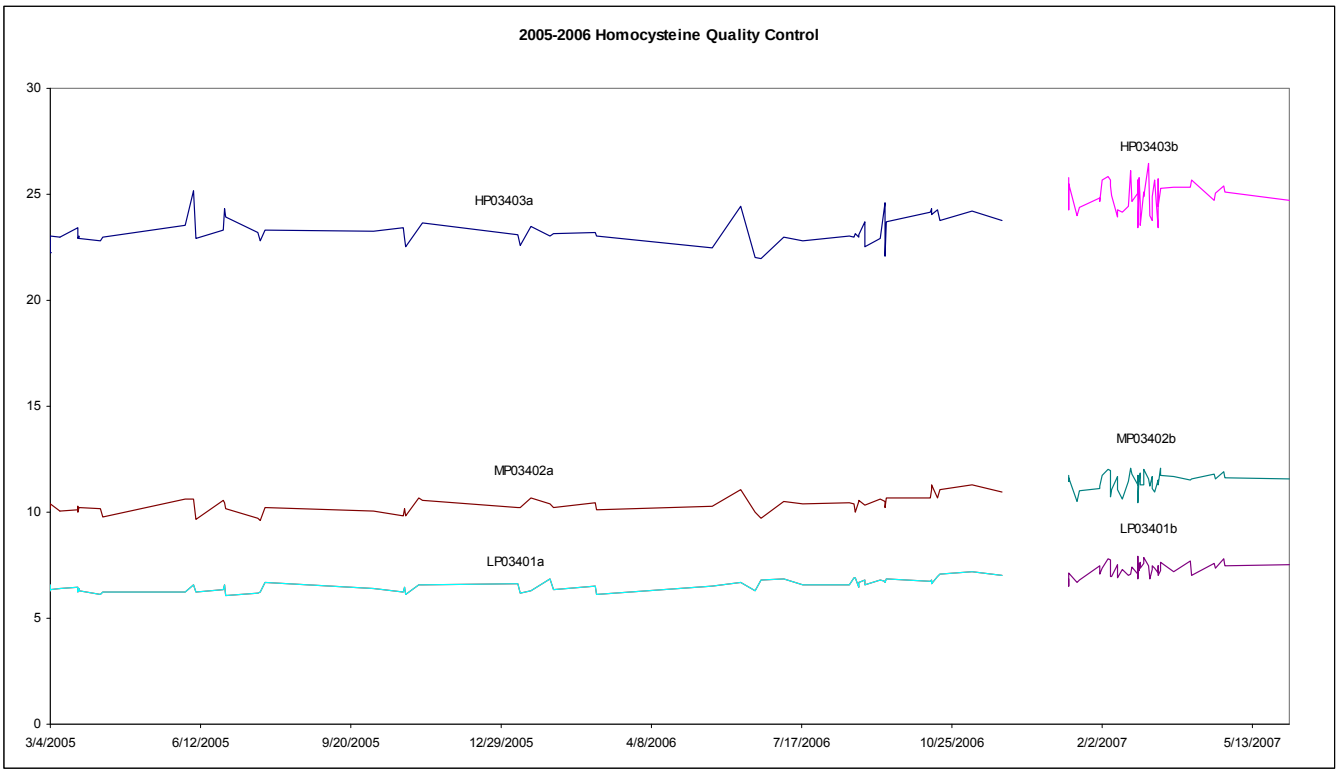
We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum 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°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
LP03401a	57	3/4/2005	11/28/2006	6.54	0.27	4.2
MP03402a	57	3/4/2005	11/28/2006	10.35	0.38	3.7
HP03403a	56	3/4/2005	11/28/2006	23.24	0.67	2.9
LP03401b	46	1/11/2007	6/7/2007	7.31	0.33	4.6
MP03402b	46	1/11/2007	6/7/2007	11.47	0.41	3.5
HP03403b	46	1/11/2007	6/7/2007	24.92	0.75	3.0



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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