Laboratory Procedure Manual

Analyte: **Glycohemoglobin**

Matrix: **Whole Blood**

Method: **Primus Automated HPLC System (Primus I, Model CLC330)**

Method No.:

Revised:

as performed by:

Contact:

Important Information for Users

The University of Missouri-Columbia 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

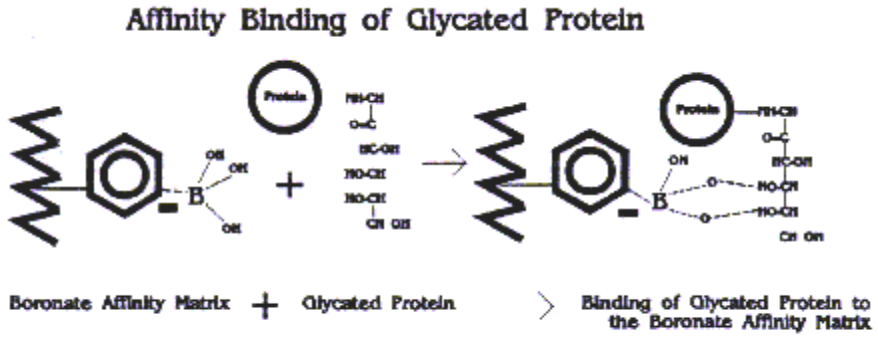
This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
I10_c	LBXGH	Glycohemoglobin (%)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Glycated proteins differ from non-glycated proteins by the attachment of a sugar moiety(s) at various binding sites by means of a ketoamine bond. Glycohemoglobin (GHb) thus contains 1, 2-cis-diol groups not found in non-glycated proteins. These diol groups provide the basis for separation of glycated and non-glycated components by boronate-affinity chromatography (1–3). In this analytical technique, a boronate such as phenylboronic acid is bonded to the surface of the column support. When a solution of proteins (e.g. hemolysate) is passed through the column, the glycated component is retained by the complexing of its diol groups with the boronate. After the unretained non-glycated component elutes from the column, the glycated component is eluted from the column with a reagent that displaces it from the boronate.



The Primus instrument is a fully automated glycohemoglobin analyzer which utilizes the principle of boronate-affinity high performance liquid chromatography (HPLC) (4). The analytical column contains aminophenylboronic acid bonded to a porous polymer support (gel). The low- and high-pressure pumps transfer reagents through the analytical column, with reagent selection executed by a switching valve. Hemolyzed samples are automatically injected onto the column during the flow of A-Elution Reagent #1. The glycated component binds to the boronate, while the non-glycated component passes through the column to the spectrophotometric detector, where it is detected at wavelength of 413 ± 2 nm. After the elution of non-glycated component, the Primus instrument pumps B-Elution Reagent #2, which displaces the glycated component from the column. The glycated component then passes through the detector. In the final stage of each sample cycle, the column is re-equilibrated with Elution A-Reagent #1. All reagent selection occurs in a timed sequence designed to allow complete elution of non-glycated and glycated components.

All functions in the liquid chromatograph and computing integrator are controlled by microprocessors (Model CLC330) or PC computer (Model CLC385). The signal from the spectrophotometric detector is processed and the concentration of glycohemoglobin is calculated as a percentage of the total detected. Integration is by peak area in millivolt-seconds.

The chromatogram is plotted first as the signal is received by the detector. The raw %GHb is calculated when glycated hemoglobin peak area is divided by the total hemoglobin peak area. Primus HPLC uses two point calibrators with HbA1c assigned values to obtain a final standardized GHb. The Schiff base does not interfere with boronate affinity method. The report is then printed with the sample information, raw GHb and standardized GHb results.

GHb is an index of average blood glucose levels for the previous 2–3 months (5, 6). It is widely used as an indicator of glycemic control in the care and treatment of patients with diabetes mellitus.

2. SPECIAL SAFETY PRECAUTIONS

Wear gloves, lab coat, and safety glasses when handling human blood specimens. Place all plastic tips, sample cups, and gloves that contact blood in a biohazard waste container. Discard all disposable glassware into a sharps waste container. Place all liquid hazardous waste materials in closed containers labeled as hazardous waste and stating the composition of waste being contained. These containers are collected and disposed of weekly by University of Missouri hazardous waste management personnel.

Protect all work surfaces with absorbent bench top paper. Discard bench top paper into a biohazard waste container weekly or whenever blood contamination occurs. Disinfect all work surfaces with Envirocide weekly.

Material Data Safety Sheets (MSDS) for human whole blood hemolysates and Envirocide are available at the Diabetes Diagnostic Laboratory, University of Missouri-Columbia.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Each NHANES IV shipment is labeled with a unique container number. An electronic shipment file is sent to the laboratory at the time when samples are shipped. This file corresponds with the Shipping Manifest Report (SMR) included in each shipment of specimens. The electronic file contains sample ID, slot ID, collection date, time, and comment code associated with each specimen. The file is formatted as a comma delimited file with a .shp extension.

B. The electronic file is saved to a network drive with a .txt extension. A backup copy is created for each file.

C. A Microsoft Access database (Hanes4.mdb) has been established on the network drive. The shipment file is first imported into a temporary import table in the database. After the data is verified with SMR, the file is then imported into Glycohemoglobin analyte table.

D. A batch number is assigned to each shipment. A unique and sequential laboratory accession number is assigned to each specimen. A blank "Data Check Sheet" (work list) is generated by batch number and by analyte for the laboratory technologists.

E. All test result and quality control (QC) files are stored on the network server. Files are backed up daily on tape and monthly on CDs.

F. Records of specimen tracking and system maintenance are kept on Sample Flow Tables located in the same database.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Samples are collected in accordance with NHANES specimen collection criteria for the glycosylated hemoglobin test. No special instructions such as fasting or special diets are required.

B. Specimen type: whole blood with anticoagulant, preferably K_3EDTA at a concentration of 1.5 mg/mL whole blood.

C. The optimal amount of specimen is 0.5 mL; minimum amount is 100 µL (0.1 mL).

D. An acceptable container is a 2-mL or larger vacuum tube (i.e., a lavender-top Vacutainer). K_3EDTA is the recommended anticoagulant. Clotted specimens are unacceptable for this test.

E. Any samples that exhibit undesirable chromatograms (i.e., high baseline) are repeated for confirmation. If the sample still shows an undesirable chromatogram, no result is reported and a comment is listed in the assay comment code field.

F. Samples are refrigerated at 4–8°C immediately after collection and transported at 4–8°C on refrigerant packing material. Once received and analyzed, samples may be frozen and stored at –70°C. Do not freeze samples at –20°C. Samples are stable at room temperature (approximately 22°C) for at least 28 days, four months at 4°C, and three years at –70°C. Avoid multiple freeze/thawing of samples (7).

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) Primus Automated HPLC system, model CLC330 (Primus I) from Primus Corp. Kansas City, MO. consists of four main components:

(a) The liquid chromatograph (LC) houses all the components involved in sample processing, including reagent pumps, autoinjector, autosampler, and photometric detector.

(b) The computing integrator processes the electronic signals from the LC, calculates test results, and prints reports.

(c) Two disk drives operate with the computing integrator. Drive A is used for the operations software; drive B is used for the test results on disk.

(d) The operation software provides automated control of the CLC330 analyzer.

Specification:

Sample capacity: 100 samples per batch, plus urgent slot position for priority sample.

Throughput period: 2.5 minutes per sample.

Minimum readable division: 0.1%

Sample requirement: 5 μL whole blood or packed cells.

Sample volume after hemolysis: 1mL

Column temperature: 40°C ± 0.5°C.

Autoinjector: Compressed air driven; delivers sample volume with <1% error; 1-25 μL injection volume capacity.

Detection: UV/Visible wavelength detector, 413 ± 2 nm; split beam photodiode referenced.

Power: AC 120V, 60 Hz, 7 Amps max.

(2) Primus Automated HPLC system, model CLC385 (Primus IV) from Kansas City, MO. consists of three main components:

(a) The liquid chromatograph (LC) houses two reagent pumps, oven for the analytical column, and the photometric detector.

(b) The computer (Pentium, 133 MHz, running Windows 3.1) controls the LC and autoinjector. The printer prints out the chromatograms and results.

(c) The autosampler/autoinjector combination injects the hemolysates into the LC component.

Specification:

Sample capacity: 112 samples per batch, plus urgent slot for priority sample.

Throughput period: 2 minutes per sample.

Minimum readable division: 0.1%.

Sample requirements: 5 μL of whole blood.

Sample volume after hemolysis: 0.5 mL.

Column temperature: 50°C ± 1°C.

Autoinjector: Mechanically driven, delivers sample volume with < 1% error; 1-25 μL injection volume capacity.

Detection: UV/Visible wavelength detector, 413 ± 2 nm; split beam photodiode referenced.

Power: AC 120V, 60 Hz. 7 Amps max.

(3) Diamat autodilutor, model AD-7 (Bio-Rad Laboratories, Hercules, CA).

Sample volume: 5–100 µL, reproducibility ≤0.5%

Reagent volume: 1000 µL, reproducibility 0.2%

Overall accuracy: 1.0%

(4) Mettler AT200 Electronic Balance (Fisher Scientific, St. Louis, MO)

B. Materials:

(1) All reagents and supplies listed below are supplied by Primus Corp (Kansas City, MO)

	Primus I (CLC330)	Primus IV (CLC385)
GHb diluent/hemolysis reagent	Ultra-pure filtered HPLC Grade water with 0.001% Sodium Azide (DIL)	Ultra-pure filtered HPLC Grade water with 0.001% Sodium Azide and detergent (DIL2).
Elution reagent #1	Acetate – Salt buffer in 5% Alcohol plus 1% Urea, pH 9.	Acetate – Salt buffer in 5% Alcohol plus 1% Urea, pH 9.
Elution reagent #2	Polyol – Salt in 5% Alcohol, pH 7	Polyol – Salt in 5% Alcohol, pH 7
Wash solution	Ultra-pure filtered HPLC grade water with 0.001% sodium azide in 5%	Ultra-pure filtered HPLC grade water with 0.001% sodium azide in 5%
Software	workdisk	Windows based program
Analytical column	aminophenylboronic acid bonded to a porous polymer support	aminophenylboronic acid bonded to a porous polymer support
Sample vials	Glass vials with caps	plastic vials without caps

(2) Additional materials used by Primus I and IV supplied by Primus Corp (Kansas City, MO)

- Printer paper
- Printhead cartridge
- Vial cap crimping tool (Primus I)
- Result disk to record patient results (Primus I)
- Autosampler racks

(3) Other materials

- Nitrogen Bottle (any vendor)
- Powder free hypoallergenic latex examination gloves (Microflex Medical Corporation, South San Francisco, CA)
- Biohazard waste storage bags and boxes (Jefferson Smufit Corporation, Highland, IL)

- Viro Research Envirocide Disinfectant Decontaminant Cleaner (Fisher Scientific, St. Louis, MO)
- Gauze Sponges 4×3 16 ply, not sterilized (Johnson-Johnson, New Brunswick, NJ)
- Transfer pipettes (Fisher Scientific, St. Louis, MO).
- Single fold paper towels (Ft. Howard Corp. Co, Green Bay, WI).
- Kim Wipe lintless tissues (Kimberly Clark Corp., Roswell, GA).

C. Storage Requirements: Reagent Use and Storage

All reagents are to be stored at room temperature, and are ready to use. In their original container they are stable until the expiration date on each bottle. Reagents #1, 2, and 3 are stable 21 days at room temperature in the instrument reagent reservoirs. Reagent reservoirs are cleaned and the reagents are replaced every 21 days.

D. Calibration Preparation:

Primus HPLC uses a two point calibration system. Initial calibrator values were based on the mean of multiple determinations from the National Glycohemoglobin Standardization Program (NGSP) (8) Central Reference HPLC method (9). This reference HPLC method measures hemoglobin A1c, a specific glycohemoglobin, and has been described previously in detail (10).

Seven 7-mL EDTA Vacutainer tubes of venous blood were drawn from five non-diabetic and five diabetic individuals. Whole blood from non-diabetic individuals was combined to make Pooled Low Calibrator 2 (PLC2). Whole blood from diabetic individuals was combined to make Pooled High Calibrator 2 (PHC2). For each calibrator, the tubes were pooled, mixed and dispensed in 25-µL aliquots and stored at –70°C. This quantity of calibrator material lasts for at least three years. Assigned values for subsequent calibrator lots are based on the mean of 20 determinations by the Primus HPLC calibrated assay method. Calibration procedures are described below.

E. Preparation of Quality Control Materials:

Seven 7-mL EDTA Vacutainer tubes of venous whole blood were drawn from five known non-diabetic individuals (WB17) and six known diabetic individuals with an elevated Std GHb level (WB18). For each level of the controls, the tubes were pooled, mixed and dispensed into 50-µL aliquots in a refrigerated room. Controls are stored at –70°C and are stable for at least three years.

F. Calibration Procedure:

Calibration is performed when a column is changed, or when controls are out of range, or when switching to a new computer disk.

(1) To calibrate the Primus I instrument:
   (a) Choose ‘5’ from the main menu. This will bring up the calibration menu.
   (b) If new calibrator values are to be used, change the values by selecting ‘6’ from the menu and enter the new values.
   (c) Choose ‘3’ (GHb Calibrator/Controls) to calibrate GHb only and run controls (to insure that the calibration is where it should be). The order of vials will be displayed.
   (d) Place the two calibrators and the two controls in their respective positions in a rack. Press Enter.
   (e) After calibration, return to the main menu by choosing ‘4’.

(2) To calibrate the Primus IV machine:
   (a) Choose ‘Calibration’ from the main menu.
   (b) Choose ‘Enter Values’ to change the values of the calibrator.

(c) Choose 'Run' to start the calibration.

(d) Place the two calibrators and the two controls in their respective positions in a rack. Press Enter.

(e) After calibration, the main menu will return.

G. Procedure Operating Instructions; Calculations; Interpretation of Results

(1) Preliminaries:

(a) All reagents should be at room temperature before assay.

(b) Allow frozen calibrators, QC specimens, and any frozen blood samples to thaw. Mix all samples at least ten minutes on a rocker before preparing them.

(c) Use a permanent marker to label sample vials with the corresponding sample identification MU accession numbers.

(2) Sample preparation:

Using the Bio-Rad autodiluter, prepare control and patient hemolysates by making a 5:1000 dilution (WB to diluent) for Primus I and 5:500 dilution for Primus IV. Use the following procedure:

(a) Make sure that appropriate volume control rod is selected for the autodiluter. Use DIL2 reagent for Primus IV and Primus I.

(b) Insert tip of autodilutor into blood specimen and press the button on top of the handle to draw 5 μL of specimen into the tip.

(c) Wipe the tip clean with wet gauze and insert the tip into the corresponding sample vial. Press button again to dispense sample and reagent into the sample vial.

(d) Repeat procedure for all NHANES specimens and QC samples.

(e) If Primus I is used, cap the vial and mix thoroughly too completely lyse cells. Primus IV vials do not require mixing.

(3) Instrument setup for the Primus CLC330 HPLC System (Primus I):

(a) Press the "System On" button on the front panel. The instrument will perform a self-test and display a series message. After a successful self-test, the following message will be displayed: "HP 1090L system with INET on".

(b) Press the power switch on the rear panel of the disk drives. Wait for the disk drive to finish its start-up routine.

(c) Press the power switch on the left rear panel of the integrator to the "On" position. When the integrator completes its self-test, "LOOP UP" will be printed.

(d) Enter the date: "DA mm/dd/yyyy".

(e) Enter the time: "TI hh:mm:ss".

(f) Enter BASIC mode by typing "BA".

(g) Set the top of the form by pressing [shift] + [Enter] until the paper perforation is about an inch above the print head. Then press [Ctrl] + [k].

(h) At the > prompt, type "run a:hplc". The integrator will print out the date and time with a banner of the program name and version of the software, the last shutdown date and time, and the methods that are available.

(i) The main menu will appear.

(j) The instrument will go through a 30 minute warm up period before any samples can be analyzed. Equilibrate the column by running four dummy samples before analyzing the first sample of the day.

(k) Place sample vials in the Primus I autosampler racks in the following order:

(l) One quality control specimen in position 1 in rack #1.

(m) Patient samples in subsequent racks with one QC specimen (alternating high and low

(n) QC) after every 19th patient specimen and at the end of the run.

(o) To shut down the CLC330, press “X” from the main menu. The system will automatically shut down at the end of a run.

(p) Batch Summary Report is automatically generated at the completion of each run. This report includes the vial number, sample ID, raw GHb results, standardized GHb results and filename. The normal range is also printed.

(q) Report any abnormal chromatograms to the supervisor for further investigation.

(4) Instrument set up for CLC385 HPLC System (Primus IV):

(a) Turn on the power to the computer and the monitor.

(b) From the menu at the top of the screen, choose “activate system” from the Main menu. The column and the system will perform a 14 minute warm-up.

(c) Check tubes for air bubbles. Purge all bubbles from the line by drawing buffer through the system using a syringe.

(d) Enter and edit the sample information using the Main menu.

(e) Place sample vials in the Primus IV autosampler tray in the following order:

(f) One non-diabetic and one elevated QC in priority area positions 1 and 2, respectively.

(g) Patient samples in sample tray starting at position 1 (maximum 112).

(h) From the computer screen, request one QC specimen (alternating high and low QC) every 20 patient specimens and also at the end of the run.

(i) To analyze samples, choose “run samples” from the main menu. The Primus IV column does not require pre-analysis equilibration.

(j) The instrument will shut down automatically if the "shut down" step has been selected prior to the completion of a run. To manually shut down Primus IV, choose the “Deactivate system” option.

(k) A Batch Summary Report is automatically generated at the completion of each run. This report includes the vial number, sample ID, raw GHb results, standardized GHb results and filename.

(l) Report any abnormal chromatograms to the supervisor for further investigation.

H. Recording of Data

(1) Quality Control Data

All replicate values of QC data plus all pertinent assay information (date of analysis, reagent lot number, technician ID, samples ID etc.) are recorded in the Microsoft Access Primus Daily Diary Log database located on the network drive. The calibrator values are also recorded.

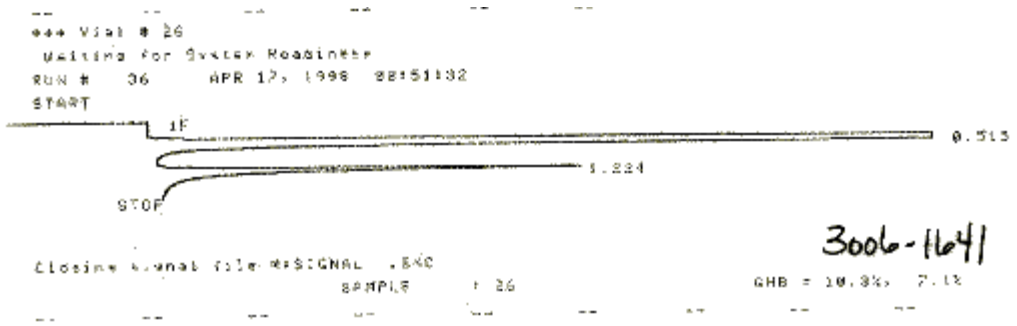
Enter the data under the form “Diary Sheet Entry Form”. The Microsoft Access program will automatically calculate the daily mean and range for each control and determine if a run is accepted or rejected. The current above or below the mean trend is also calculated. The program will print out a diary sheet for each run and the information is checked and signed by a supervisor.

(2) Analytical Results

(a) After run is completed, mark sample ID numbers on the individual chromatograms. Match the position of each vial in the autosampler rack to the sample number printed on each chromatogram.

(b) Examine the chromatogram carefully. The first peak (non-glycated) is quite large compared to the second (glycated) peak, so only part of the first peak appears on the chromatogram. If the first peak is not flat on the top, it indicates that the hemolysate hemoglobin concentration is too low. Prepare a more concentrated hemolysate from the original specimen and repeat the analysis.

(c) The second peak (glycated) should be clearly resolved. There should be a smooth curve between the peaks. The trough between peaks should be above the baseline.



(d) The first reported GHb value is a raw value (without calibration), the second value is the calibrated or the standardized GHb (std. GHb).

(3) Record the %Std GHb results onto the "Data Check List", matching the UMC accession numbers on the batch report with the corresponding numbers on the data check list.

(4) GHb results are entered in Hanes4.mdb database. During the data entry process, check the lab accession numbers.

(5) NHANES IV has established a list of assay comment codes for reporting results. If a result is below the assay detection limit, or a sample is missing, or if the sample volume is less than 50 µL, leave the result field blank and record an appropriate comment code in the assay comment field.

(6) A second Data Check Sheet List with test results is printed. Test results are verified against the instrument print out. A copy of the data check sheet is kept in the NHANES IV GHb Data Book at the Diabetes Diagnostic Laboratory at the University of Missouri.

(7) A comma delimited text file (container id.txt) is generated in Hanes4.mdb with an export query. The file follows the format specified by NHANES IV. A copy of the text file is printed and the information is validated against the data check sheet.

(8) The data files are exported by batch within three weeks of reception of the specimens. The text file is sent via electronic mail to Westat.

(9) The quality control information and the assay information are entered into the Microsoft Access Primus Diary Log Sheet database located on the network drive. A QC file (mmyyGHB.txt) is generated from the Primus Diary Log Sheet database following the format specified by NHANES IV. The file is sent monthly to Westat via electronic mail.

I. Equipment Maintenance:

(1) Primus I System – Routine maintenance:

(a) Analytical Column – reverse the column every 500 injections. Change the column whenever signs of column deterioration are evident (increase in column pressure, loss of peak resolution). At least two spare columns are to be kept available at all times.

(b) Record all routine maintenance in the Primus Maintenance Diary Log.

(2) Primus IV System – Routine maintenance:

(a) Frits – change frits going out from pump A and pump B every 1500 injections. The frit going out from the injector needs to be changed after 3000 injections.

(b) Analytical column – reverse the column every 500 injections. Change the column whenever signs of column deterioration are evident (increase in column pressure, loss of peak resolution.

(c) Purge all air bubbles from the lines by drawing buffer through the system using a syringe.

(d) Record all routine maintenance in the Primus Maintenance Diary Log.

(3) Primus Systems – Periodic/Preventative maintenance – Preventative maintenance inspections are to be performed yearly by Primus Corporation (Kansas City, MO).

(4) Bio-Rad Autodilutor – Calibrate and check syringes for leakage every six months.

(5) Mettler AT200 Electronic Balance – Although the unit is self-calibrating, it is to be checked and verified for proper operation annually by an authorized service technician.

(6) Pipettes (Bio-Rad Autodilutor and Rainin digital pipettes) are to be calibrated every six months following the Diabetes Diagnostic Laboratory Standard Operation Procedures.

(7) Temperatures of all refrigerators / freezers used to store specimens, controls and calibrators are monitored with "7 day/24 hour" type of temperature recorders. Any readings that fall outside the acceptable limits are reported to the supervisor for corrective action. The temperature charts are checked, reviewed and signed weekly by a supervisor.

Calculations:

(a) All calculations are performed by the Primus HPLC system.

(b) Calculation of the percentage of GHb in the sample is by the following formula:

$$\frac{\text{Area of Peak 2 (GHb)} \times 100}{\text{(Area of Peak 1 (Non-GHb) + Area of Peak 2 (GHb))}}$$

(c) Retest any specimens with % Std GHB values less than 4.0 % or greater than 14%.

9. REPORTABLE RANGE OF RESULTS

Std GHB results are reported throughout the range of 2.0–20.0%. Results above 14% or below 4.0% are repeated for verification prior to being reported.

10. QUALITY CONTROL (QC) PROCEDURES

Two types of quality control systems are used in this method: 1) "batch QC" specimens that are placed in each run, and 2) "sample QC" specimens (2% of the total specimens) that are randomly selected from each run and analyzed in another run. If the coefficient of variation between duplicates is greater than 10%, the specimen is reanalyzed and the chromatograms, instrument, and QC data from both the original and duplicate runs are investigated before the results from the original run are reported.

The batch QC consists of two levels of frozen whole blood controls. Values are in the near normal (WB17) and elevated (WB18) GHb ranges. Daily means and ranges are calculated from 20 interassay determinations. The bias ranges of the daily means are set at ±1 SD or the 67% confidence interval (CI); the warning limits (WL) are the ±2 SD or the 95% CI and the control limits (CL) are the ±3 SD or the 99% CI. For the daily ranges, the bias limit is the mean + 1 SD with warning and control limits set at the mean +2 SD and the mean + 3 SD, respectively.

Table 5 shows the precision and accuracy as demonstrated by representative pools used during this survey.

Table 5. Precision and Accuracy

Pool	Mean	95% limits	99% limits	Runs	CV (%)	95% range	99% range
WB17	5.1	4.9-5.3	4.8-5.4	20	2.54	0.3	0.4
WB18	9.9	9.7-10.1	9.6-10.2	20	0.90	0.3	0.4

Two types of long-term QC charts (11, 12) are used. The first type of chart plots the mean values for each run. The system is declared "out of control" if any of the following conditions occur:

- The mean for one control from a single run falls outside the 99% confidence limits.
- The means for two controls from a single run fall outside the 95% confidence limits.
- The daily means for one control from eight successive runs (excluding runs in which the mean is within 1 SD or bias range) fall either all above or all below the center line.

The second type of QC chart plots the range of the replicates (the difference between the highest and the lowest value for each control within a run) and compares with the established target ranges, which is the overall mean of daily ranges established by 20 characteristic runs. The system is declared "out of control" if any of the following conditions occur:

- The range from a single run for a single control falls above the upper 99% confidence limit.
- The ranges from a single run for both controls fall above the upper 95% confidence limit.
- The ranges from eight successive runs (excluding the runs in which the mean is within1 SD or bias range) are all above the mean line.

If a run is declared "out of control," all patient samples from that run are re-analyzed in subsequent runs. Additionally, the instrument, standard, and controls are investigated to determine the cause of the problem before further analysis occurs.

The UMC laboratory participates in an external QC program sponsored by the College of American Pathologists (CAP). Fresh blood specimen is received quarterly from CAP, reconstituted, and analyzed. Results are submitted to CAP, where the %Std. GHb value is compared with consensus mean, low, and high %Std. GHb values obtained from participating laboratories using the same assay method. A copy of the CAP report is received by the UMC laboratory and is reviewed and approved by the supervisor.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

A. If the system is declared "out of control" for any of the reasons listed in Section 10 of this manual, take the following steps:

(1) Examine chromatograms from the run for any abnormalities (e.g., peaks coming off the column too quickly or too slowly, poor resolution of peaks, extra peaks). Poor resolution of peaks may indicate the need to change the analytical column. For other problems, consult with the supervisor for appropriate corrective action.

(2) Prepare fresh standards and controls and re-analyze freshly diluted samples in a different run.

(3) Document the problem and any actions taken on the daily diary log sheet.

B. If the above steps do not correct the "out of control" condition, consult with the supervisor for further corrective actions. Do not report any values from runs that are not accepted.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Patients with hemolytic anemias may exhibit decreased Std GHB values because of a shortened red cell life span. Patients with polycythemia or postsplenectomy may exhibit increased Std GHB values because of a somewhat lengthened red cell life span (13, 14).

B. Test results are not affected by lipemic or hemolyzed samples.

13. REFERENCE RANGES (NORMAL VALUES)

The normal range for the HbA1c test was established in-house. The mean %HbA1c value for 181 nondiabetic subjects collected from throughout the continental United States was 5.0%, with a 99% CI of 4–6%. Subjects with fasting glucose greater than 110 mg/dL were excluded from the calculation (15).

14. CRITICAL CALL RESULTS (PANIC VALUES)

A. Std. GHb = 7%.

B. Medical intervention may be necessary. Subjects with GHb values above these specified limits are reported weekly by facsimile to National Center for Health Statistics (NCHS).

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be refrigerated at 4–8°C prior to preparation. Specimens are returned to 4–8°C immediately after preparation and frozen at –70°C after results are processed.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If the Primus Analyzer system fails, specimens are to be stored at –70°C until the system resumes operation.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

NCHS is notified weekly by facsimile of all subjects with GHb values in the diabetic ranges. The supervising physicians are then notified by NCHS.

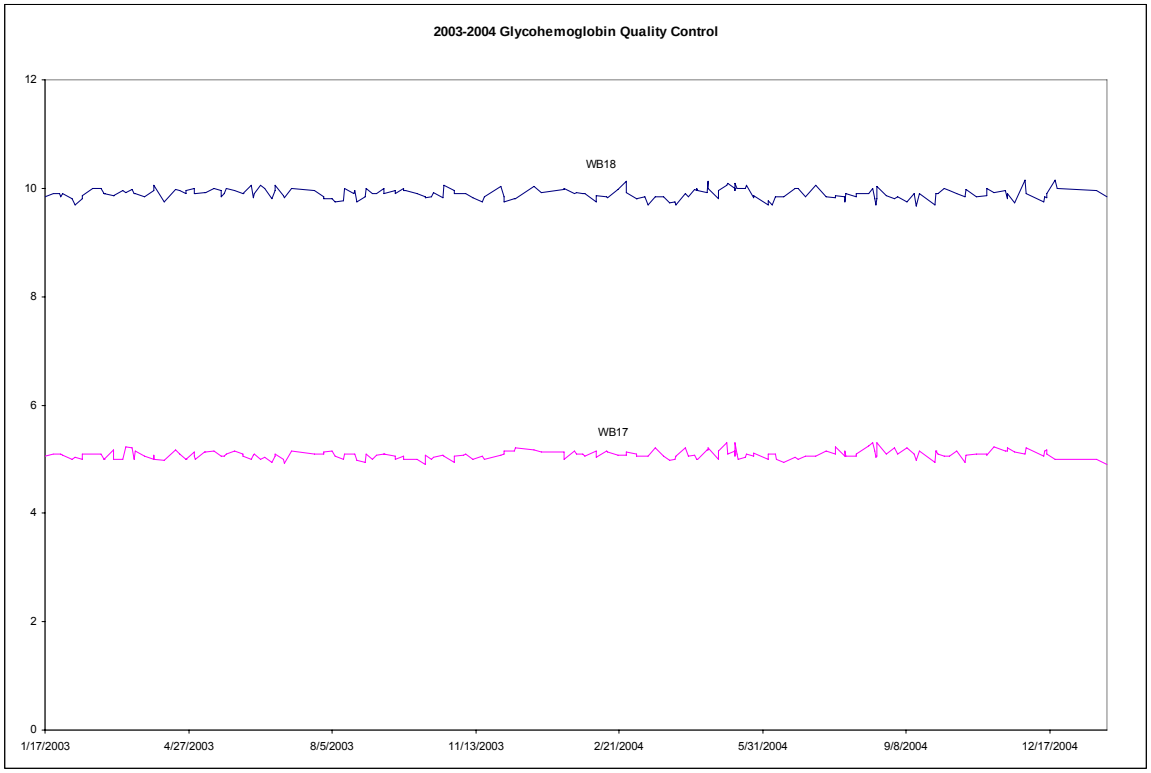
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

All specimens are tracked on both laboratory log books and electronic data files kept on the Diabetes Diagnostic Laboratory server system and back up CDs. Hard copies of all shipping manifest reports, and data check lists containing the specimen information, test results, and daily assay information is kept in 3-ring binders. The QC diary log sheet data are stored in a separate notebook. Only the NHANES ID numbers are known to the laboratory. Other personal identifiers are not provided to the laboratory in order to protect the confidentiality of study participants.

Residual samples are stored at –70°C for 1 year and periodically shipped to the NCHS serum repository in Rockville, MD.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Glycohemoglobin by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
WB17	198	1/17/2003	1/26/2005	5.08	0.08	1.5
WB18	198	1/17/2003	1/26/2005	9.90	0.10	1.0



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