Laboratory Procedure Manual

Analyte: **Plasma Glucose**

Matrix: **Serum**

Method: **Enzyme Hexokinase (HK)**

Method No.:

Revised:

as performed by: *Department of Child Health*
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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
l10am_c	LBXGLU	Glucose (mg/dL)
	LBXGLUSI	Glucose (mmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The enzyme hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). In the presence of nicotinamide adenine dinucleotide (NAD), G-6-P is oxidized by the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NADH). The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm. (1–3).

Glucose is the major carbohydrate present in the peripheral blood. Glucose derived from dietary sources is either oxidized to provide energy or converted to glycogen or fatty acids for storage in the liver and tissues. The most frequent cause of hyperglycemia is diabetes mellitus. Some other factors that contribute to elevated blood glucose are pancreatitis, pituitary or thyroid dysfunction, renal failure, and liver disease. Hypoglycemia is less frequently observed, but is found in conditions such as insulinoma, hypopituitarism, neoplasms, or insulin-induced hypoglycemia. (4)

2. 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 sharps waste containers. These containers are collected and disposed of twice weekly by University of Missouri waste management personnel.

Protect all work surfaces with disposable absorbent bench top paper, which is discarded into biohazard waste containers weekly, or whenever blood contamination occurs. Wipe all work surfaces with Envirocide solution weekly.

> Dispose of all biological samples and diluted specimens in a biohazard waste container at the end of the analysis.

Material safety data sheets (MSDSs) for Envirocide are available at Diabetes Diagnostic Laboratory at the University of Missouri, Columbia (UMC).

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 the Glucose 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 are kept on Sample Flow Tables located in the same database.

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

A. Fasting glucose samples are collected for glucose analysis following the NHANES IV sample collection protocol.

B. Specimen type: 1.5 mL plasma with NaF as preservative.

C. Optimal amount of specimen required is 1.5 mL; minimum is 0.2 mL.

D. 3–5 mL of whole blood is collected in a vacuum tube containing the glycolytic inhibitors potassium oxalate and sodium fluoride (e.g., gray-top Vacutainers). Specimens are centrifuged immediately at 1500 × *g* for 10 min. Plasma is transferred to a 2-mL cryogenic screw-cap vial and frozen at –70°C. Frozen plasma specimens are shipped weekly in batches in Styrofoam-insulated shipping containers with dry ice to the University of Missouri Diabetes Diagnostic Laboratory via over-night courier.

E. Upon receipt, all specimens are stored in a –70°C freezer until analysis. Specimen stability has been demonstrated for 1 year at –70°C. Multiple freeze-thaw cycles should be avoided.

F. The criteria for unacceptable specimens are either a low volume (<0.2 mL) or gross hemolysis. Specimens collected without NaF or those that arrive thawed are also unacceptable.

G. Specimens that do not meet the acceptable criteria are not analyzed. The reasons are noted in the assay comment codes.

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. Equipment and Instrumentation

(1) Instrumentation Cobas Mira Chemistry System (Roche Diagnostic Systems, Inc., Montclair, New Jersey).

(2) Sorvall Model GLC-2B general purpose centrifuge (DuPont Instrument, Newton, CT).

(3) Jouan refrigerated centrifuge model GR4-22 (Winchester, VA). Temperature range: –8 to 60°C, temperature accuracy: ≤2°C, maximum RPM: 8,000 maximum, timer range: 0–99 min in 1-min increments.

(4) Milli-Q Plus ultra-pure water system (Millipore, Bedford, MA).

(5) Gilson Pipetman adjustable pipette, 200- to 1000 µL (Rainin Instrument Co., Woburn, MA).

(6) Thermolyne Varimix mixer (Thermolyne Inc., Dubuque, IA).

B. Other Materials

(1) Glucose reagent (Roche Diagnostic Systems, Indianapolis, IN).

(2) Glucose standard (Beckman Instruments, Inc., Palo Alto, CA).

(3) Pipette tips, 200- to 1000-µL sizes (Fisher Scientific, St. Louis, MO).

(4) Pipette-Aid (Drummond Scientific Co., Broomail, PA).

(5) Pyrex 20-mL disposable pipette (Fisher Scientific, St. Louis, MO).

(6) 5-mL class "A" volumetric pipette (Fisher Scientific, St. Louis, MO).

(7) The following items are all supplied by Roche Diagnostic Systems, Inc. (Indianapolis, IN): sample cups, thermal paper, reagent probe, 1000-µL reagent syringe, replacement plunger tip for both reagent and sample syringes, sample needles, reagent containers, cuvette segments, sample racks, calibration rack, reagent rack, and thermal printer paper.

(8) Disposable gloves (Fisher Scientific, St. Louis, MO).

(9) Biohazardous waste storage bags and boxes (Fisher Scientific, St. Louis, MO).

(10) Absorbent bench top paper (any vendor).

(11) Bleach (10% sodium hypochlorite solution) (any vendor)

(12) Viro Research Envirocide Disinfectant Decontaminant Cleaner (Fisher Scientific, St. Louis, MO).

(13) 1.8 mL Nalgene cryogenic Vials (Nalgene Company, Rochester, NY).

(14) Lyophilized serum controls (Bio-Rad Laboratories, Richmond, CA).

(15) NIST SRM909 lyophilized serum reference material (National Institute of Standards and Technology, Gaithersburg, MD) .

C. Reagent Preparation

Glucose Reagent

Roche reagent for glucose (Roche Cobas catalog # 47383) is ready to use as supplied. No preparation is necessary. If more than one vial of reagent is needed, solutions from different vials with identical lot numbers may be pooled and mixed prior to analysis.

Glucose reagent is stable until the expiration date on the label when stored tightly capped at 2–8°C. When the reagent is used at room temperature, the on-board stability is 10 days. The reagent should be clear and colorless. Turbidity may be a sign of contamination. Any turbid reagent should be discarded.

D. Standards Preparation

(1) Glucose standard

(2) 150 mg/dL (Beckman , Fullerton, CA), 100 mg/dL and 500 mg/dL (Sigma, St. Louis, MO).

(3) Calibration standard

150 mg/dL of Certified Glucose (D-glucose)

(4) Standard Solution is purchased from Beckman Company. The standard is calibrated against the National Institute of Standards and Technology (NIST) standard reference material SRM 909. Store the standards in 0.5 mL-aliquots in tightly capped storage tubes at 4°C until the day of assay. A well mixed standard is placed at the cup position 1 in the calibration rack and allowed to reach room temperature. A new calibration standard tube is used for each assay. Stable until expiration date.

E. Preparation of Quality Control Materials

The two commercial lyophilized serum controls BR3 (Lot 15090, Level 1) and BR4 (Lot 15090, Level 2) are purchased from Bio-Rad Laboratories (Irvine, CA). Tap the vial gently to dislodge lyophilized cake. Using a volumetric pipette, reconstitute each vial with 5 mL of distilled water. Allow the vials to stand at 20-25°C for 30 min and then swirl gently to mix contents. Invert the vial several times until the contents are completely dissolved. Store tightly capped vials at 70°C. A new tube is used for each assay. Stable until expiration date.

Two in-house controls, IHH5, and IHL5, were prepared by collecting 450 mL (one unit) of whole blood from two diabetic and two nondiabetic subjects. The blood was collected in blood bags containing EDTA as an anticoagulant. The plasma was separated immediately from the red blood cells by centrifugation in a refrigerated centrifuge (4°C) for 25 min at 1500 × *g*. The plasma was then removed from the red blood cells, aliquoted in 0.5-mL portions and stored at –70°C in Nalgene cryogenic vials. One vial of each control is thawed and used in each assay. Reconstitution is not required for the in-house controls.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve

(1) The Cobas Mira glucose assay uses a single 150-mg/dL calibration point, which is usually analyzed in each run as a sample. The glucose value for the calibrator should be set within limits of 147–153 mg/dL. The instrument requires recalibration if the value of the calibrator is outside the specified limits.

(2) To calibrate the instrument, place a well-mixed 150 mg/dL glucose standard in Calibrator Cup Position 1 on the Calibration Rack and work under the ROUTINE work list. Choose "CA" for the sample position. The screen will respond with "CAL." The cursor/highlighter will move to accept the test entry.

(3) Select GLUCOSE for the test key, and press ENTER.

(4) The calibration entry will disappear, but the procedure is programmed and the calibration will be performed automatically.

(5) Recalibration of the instrument is also performed when QC results fail to meet the acceptable criteria and/or a new lot of reagent is used (See Sections 10 and 11.).

B. Verification

(1) In order to verify the calibrator, use Sigma glucose (D-glucose) standards (100 and 500 mg/dL) purchased from Sigma Chemical Company. The standards were calibrated against NIST standard reference material SRM 909, and are stable until expiration date.

(2) Analyzed these standards in a routine assay. Agreement with certified values should be 5%. Frequency of verification is quarterly or whenever it is necessary for troubleshooting the system.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

(1) For information regarding the range of linearity and how to handle results outside this range, refer to the Calculations section below.

(2) Allow frozen plasma samples, quality control specimens, and the glucose calibration material to reach

(3) 20–25°C and mix on a Varimix mixer 8–10 times.

(4) While specimens are thawing, allow the glucose reagent to reach 20–25°C.

(5) Use a fine-point permanent marker to label the sample cups in each sample rack with the UMC ID numbers corresponding to the specimens to be analyzed.

(6) Check the reservoir and waste bottle level. Fill or empty the bottles as needed. Only Type II reagent grade water is used in the reservoir bottle.

(7) Fill the analyzer with clean cuvette segments. Press down the segments and be sure that they are seated properly.

B. Sample preparation

(1) To prevent fibrinogen from clogging in the sample pipetting system, centrifuge specimens at 1500 × *g* for 10 min prior to analysis.

(2) Using a Gilson Pipetman, transfer 300 µL of controls and samples into the corresponding sample cups. Close the caps tightly and load them into the sample racks following the sample positions in the order set-up in the work list.

C. Instrument setup

(1) Under PROG, set up system parameters and instrument configuration as shown in Table 1.

(2) Press the ON switch on the Cobas Mira analyzer.

(3) Under INFO and SYSTEM CHECKS, prime the tubing and syringe with water.

(4) Streams from both sample and reagent probes should be straight and continuous. The syringes should be free of air bubbles.

(5) Observe the sample needle and reagent probe. They should not appear damaged. Replace them if necessary. Check that the sample tubing is seated properly.

(6) Use the PROG section to check the glucose test profile. The current list for the glucose profile is shown in Table 1.

Table 1. Cobas Instrument Parameters

	Parameter	Setting
Analytical Parameters	Temperature	37°C
	Operation Mode	Sample Selective
	Control Interval	Each Day
	Time	No
Output Mode (Printer)	Status	On
	Number of Copies	1
	Sample Auto Mode	SPL/CAL/CS
	QC Auto Mode	OFF
Instrument Configuration	Software-Version	8735C
	Interface	Installed
	DENS/Quality	Installed

D. Operation of Assay Procedure

(1) The in-house control pools IHL5 and IHH5 are placed in cup positions 1 and 2, the calibrator is placed in cup position 3 and the two commercial control pools BR3 and BR4 are placed in cup positions 4 and 5 on the first sample rack. One of the four controls will be placed in the first cup position for each of the subsequent sample racks. There will always be one normal and one elevated control at the end of each run.

(2) Program the sample work list under ROUTINE menu. Enter the first and the last numbers of the specimens to be analyzed, and press the test GLUC. The work list will appear on the screen.

(3) A sample rack always has at least two controls, alternating between high- and low-level levels.

(4) A run always ends with the two in house controls.

(5) All sample cups are labeled with their corresponding UMC accession numbers.

(6) Verify the specimen identification numbers on the vial against the work list.

(7) Place the reagent into the appropriate positions on the reagent rack.

(8) Place the calibration rack, the reagent, and the sample racks on the rack platform.

(9) Lift the analyzer cover. Insert the empty cuvette segments into position. Press the segments down firmly. Close the analyzer cover.

(10) Start the analysis by pressing START.

(11) Press the STATUS screen to display TRANSFER and ANALYZER operation status. The status screen will indicate the appropriate times when rack and segment handling are allowed during analysis.

Table 2.Glucose Test Profile

Parameter	Setting	Parameter	Setting
Measurement Mode	Absorb	Test Range Low	0.0 mg/dL
Reaction Mode	R-S	Test High	600.0 mg/dL
Calibration Mode	Calibrator	Normal Range Low	60.0 mg/dL
Reagent Blank	Reag/Dil	High	120.0 mg/dL
Cleaner	No	Number of Steps	1
Wavelength	340 nm	Calculation Step A	Endpoint
Decimal Position	1	Reading, First	T1
Unit	mg/dL	Reading, Last	6
Analysis		Calibration Interval	On Request
Sample Diluent Name	H_2O	Reagent Blank Low	-0.0270 Å
Post Dilution Factor	2.00	Reagent Blank High	0.2700 Å
Conc. Factor	2.00	Blank Low	-0.0600 Å
Sample Volume	3.0 µL	Blank High	0.0600 Å
Cycle	1	Calibrator Cup Pos.	1
Dilution	50.0 µL	Calibrator 1	150.0 mg/dL
Reagent Volume	200 µL	Replicate	Duplicate
Calc. Sample Limit	0.2400 Å	Deviation	5.0%
Point	T1		
Reaction Direction	Increase		
Check	On		
Conversion Factor	1.00000		
Offset	0.00000		

(12) When the analysis is complete, glucose results are printed automatically on the printer tape.

(13) Discard the used cuvette segments, reagent, and sample cups in the appropriate waste container.

(14) Turn off the instrument.

E. 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 on the Microsoft Access Glucose Daily Diary Log database located on the network drive. The calibrator value is also recorded.

(2) 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.

(3) Analytical Results

Record the glucose results in mg/dL onto the "Data Check List", matching the UMC accession numbers on the instrument print-out tape with corresponding numbers on the data check list.

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

NHANES IV has established a list of 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 200 µL, or the sample is grossly lipemic or grossly hemolyzed, leave the result field blank and record an appropriate comment code in the assay comment field.

(5) A second Data Check 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 Glucose Data Book at the Diabetes Diagnostic Laboratory at the University of Missouri.

(6) 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 data check sheet.

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

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

F. Replacement and Periodic Maintenance of Key Components

(1) Perform tube cleaning and syringe priming procedure on the day of assay. Use 10% bleach for cleaning solution. Select the TC test file for the procedure. After tube cleaning, prime the syringes for 5 min with Type I or II reagent grade water.

(2) Perform precision tests monthly and after any maintenance on the sample or reagent pipetting pathway. Two different concentrations of potassium dichromate are used for the precision testing. The P150 and P250 precision tests check the pipetting precision at two different sample volumes and two different reagent volumes. The expected coefficient of variation for the P150 precision test is 1.5%. The expected coefficient of variation for the P250 precision test is 2.5%. (See instrumentation manual.)

(3) Replace both the 100-µL sample syringe and the 1000-µL reagent syringe plunger tips as needed to ensure good pipetting precision.

(4) Replace the reagent probe, sample needle, and sample tubing loop as needed.

(5) Performance verification inspections are performed by the Roche Service Engineer yearly as part of the routine preventive maintenance.

G. Calculations

(1) The Cobas Mira glucose analysis is linear up to plasma glucose concentrations of 600 mg/dL. Reanalyze samples containing more than 600 mg/dL by diluting the specimen two-fold (1+1) with distilled water. The result output must then be multiplied by 2 to account for the dilution.

(2) The detection limit, based on 10 repeat measurements of zero standard (Type II reagent grade

water) and serial dilution of a sample with a low glucose concentration, is 2 mg/dL.

(3) Glucose values less than 50 mg/dL or greater than 600 mg/dL are considered abnormal and analysis must be repeated for confirmation.

9. REPORTABLE RANGE OF RESULTS

Plasma glucose values taken at fasting are reportable in the range 2–600 mg/dL without dilution. If a plasma glucose value is less than 2 mg/dL, reanalyze for confirmation. If the result is validated, leave the result field blank and record the comment code as below the lower detection limit. If a result is greater than 600 mg/dL, the specimen should be diluted (1+1) with water and reanalyzed.

10. QUALITY CONTROL (QC) PROCEDURES

Two types of quality control (QC) systems are used in this analytical method: 1) "sample QC" and 2) "batch QC." For sample QC, 2% of specimens are randomly selected and analyzed either within-assay or between-assay for quality assurance purposes. If the coefficient of variation (CV) between duplicates is greater than 5%, the specimen is reanalyzed. Batch QC specimens are placed in the calibration rack at the end of each sample rack the entire run.

The batch QC pools consist of four levels of control pools, which cover the full range of plasma glucose concentrations for normal and diabetic populations. Two are commercial lyophilized serum controls, BR3 and BR4, purchased from Bio-Rad Laboratories (Irvine, CA).

Two other controls, IHH5 and IHL5, are prepared in-house and stored at –70°C. One vial of each is thawed and used in each assay. Reconstitution is not required for the in-house controls. All four levels of controls are assayed at the beginning and end of each analytical run. One of the in-house controls is assayed at least once in each sample rack.

If the stock of these controls becomes low, another batch is ordered or prepared in time to analyze it concurrently with the current QC materials. The new controls are used only after their means and the ranges have been established by performing 20 characterization runs.

Daily means and ranges of the controls 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. An example of the precision and accuracy for the controls used for NHANES specimens is shown in Table 3.

Table 3. Precision and Accuracy

Pool	Mean	95% limits	99% limits	95% limits (range)	99% limits (range)	Runs	Total CV, %
BR3	83.1	80-86	79-87	5.5	7.5	20	1.6
BR4	273.4	265-282	261-286	12.3	16.3	20	1.5
IHL5	63.7	61-66	60-68	3.2	4.2	30	1.9
IHH5	306.6	294-319	288- 325	11.7	15.2	30	2.0

After each assay run, all control data are recorded on the Daily Diary Log Sheet. The results of the analysis are accepted or rejected according to the guidelines established by NHANES.

Two types of QC charts are used in assessing the quality of an assay. The first chart plots the mean of all the replicate determinations in a run and compares it with the established target mean, which is the overall mean established by the 20 characteristic runs.

The NHANES guideline declares a system as "out-of-control" if any of the following events 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 the runs in which the mean is within ±1 SD or the 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 of a single control within a run) and compares it with the established target range, which is the overall mean of daily ranges established by the 20 characteristic runs.

The NHANES guideline declares a system as "out-of-control" if any of the following events occur:

- The daily range for one control exceeds the 99% confidence limit.
- The daily ranges for two controls exceed the 95% confidence limits.
- The daily ranges for one control from eight successive runs (excluding the runs in which the mean is within1 SD or bias range) are all above the mean line (trend rule).

If a run is declared out of control, investigate the system (instrument, standards, controls etc.) to determine the cause of the problem. Do not perform any analysis until the problem has been resolved.

The Diabetes Diagnostic Laboratory participates in an external QC program conducted by the College of American Pathologists (CAP). Two levels of survey materials are analyzed 3 times a year for glucose in a routine run, and results are submitted to CAP for inter-laboratory comparison.

The Laboratory also participates in a second external QC program (Unity) offered by Bio-Rad Laboratories. The individual control values obtained in all glucose assays performed each month are submitted to Bio-Rad. These values are then compared with our own cumulative mean as well as the group cumulative mean (grouped by method). Up to 12 months of statistical data is available in each monthly report. The report from both external QCs are reviewed and approved by the supervisor.

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

A. When QC results fail to meet the acceptable criteria, check the sample cup containing the QC specimen for bubbles and reanalyze the QC specimen.

B. If the QC results meet the acceptable criteria, accept the run and report the results.

C. If steps a and b do not result in correction of the "out-of-control" values for QC materials, perform precision testing and replace the syringe plunger tips as needed.

D. Recalibrate the system using a new vial of glucose standard.

E. Reanalyze the calibrator, controls, and specimens. Specimens are stable at 4-8 ^{o}C overnight. If the system requires more than 24 hours before it can be restored to functionality, use new aliquots of standard, controls, and specimens for analysis.

If the above steps do not correct the "out of control" condition, consult with the supervisor for further corrective action. Do not perform glucose analysis until the system is declared "in-control" again.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

An extensive list of drugs and other factors that may interfere with the determination of glucose is reported by Young et al (5). Examples are improper collection technique, alcohol ingestion, and bilirubin.

13. REFERENCE RANGES (NORMAL VALUES)

A. Other references: In a study of 83 males and 64 females, from the New York metropolitan area,

researchers using the Roche Reagent for Glucose on the Cobas Mira found the expected values to be 64–112 mg/dL.

B. Reference range values were selected by NHANES to be 60–110 mg/dL. (6) They are slightly different from the values (72–111 mg/dL) established in the Diabetes Diagnostic Laboratory.

Table 4. NHANES Selected Reference Range (mg/dL)

	Fasting	DDL
Mean		92
Range	60–110	72–111

14. CRITICAL CALL RESULTS (PANIC VALUES)

A. Fasting specimen 126 mg/dL. (6)

B. Medical intervention may be necessary. Subjects with glucose values above these specified limits are reported weekly by facsimile to NCHS.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are thawed and maintained at 20–25°C during analysis. Specimens are returned to storage at –70°C as soon as the analyses are completed.

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

If the analytical system fails, specimens are to be stored at 4–8°C (refrigerated) until the Cobas Mira system located in the Special Chemistry Laboratory, Department of Pathology is available for analysis. If long-term interruption (more than 24 hours) is anticipated, specimens are to be stored at –70°C.

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

NCHS is notified weekly by facsimile of all subjects with glucose values in the diabetic ranges specified by American Diabetes Association. (6) The supervising physicians are then notified by NCHS.

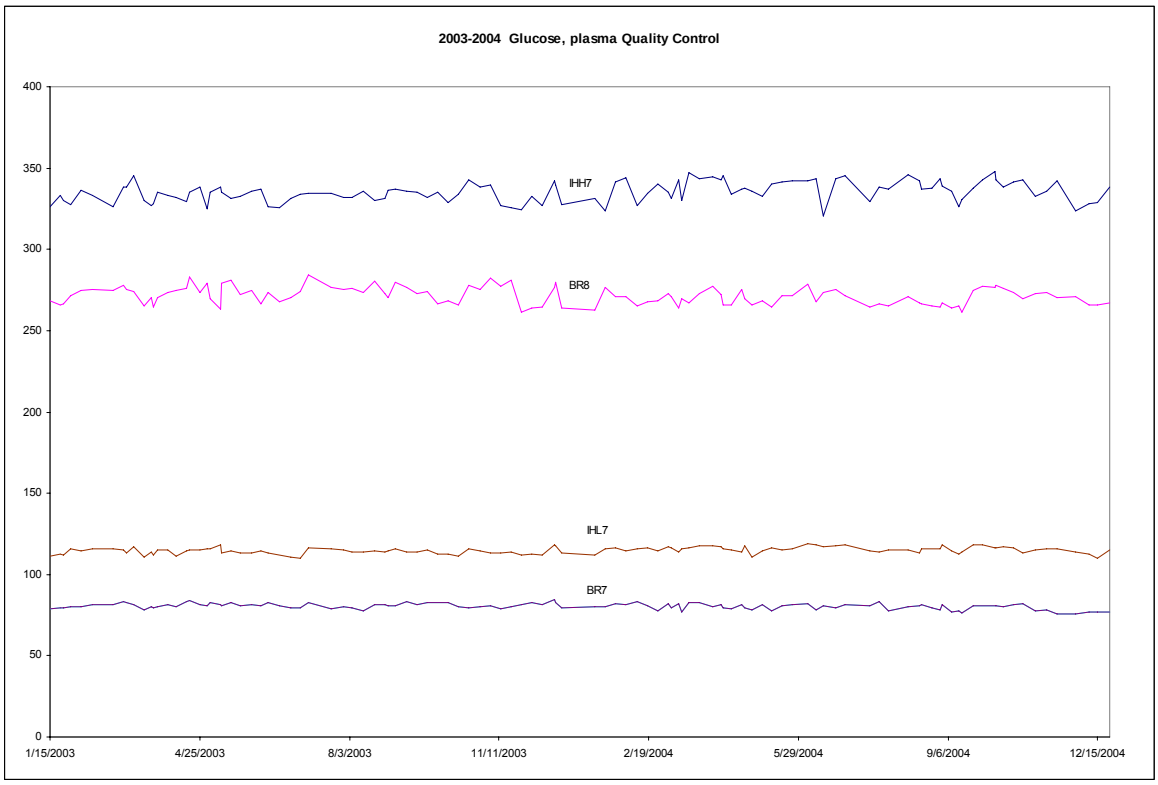
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

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 the 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 and periodically shipped on dry ice to the NCHS serum repository in Rockville, MD.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Glucose by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
BR7	112	1/15/2003	12/23/2004	80.44	1.85	2.3
IHL7	112	1/15/2003	12/23/2004	114.76	2.00	1.7
BR8	112	1/15/2003	12/23/2004	271.42	5.35	2.0
IHH7	112	1/15/2003	12/23/2004	335.23	6.17	1.8



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