Laboratory Procedure Manual

Analyte:	Fasting Glucose
Matrix:	Plasma
Method:	Hexokinase-mediated reaction
as performed by:	*University of Minnesota Medical Center, Fairview* *Collaborative Studies Clinical Laboratory* *Minneapolis, Minnesota*
Contact:	Dr. Michael Steffes

January 2008

Important Information for Users

The University of Minnesota 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 testing items in the following table:

Data File Name	Variable Name	SAS Label
GLU_D	LBXGLU	Fasting glucose(mg/dL)
	LBDGLUSI	Fasting Glucose (mmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Glucose measurements are used in the diagnosis of carbohydrate metabolism disorders such as diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia and pancreatic islet cell carcinoma.

Glucose concentration is determined by a hexokinase-mediated reaction, leading to the measurement of NADPH at 340 nm. It is an endpoint method with a sample blank measurement.

2. SAFETY PRECAUTIONS

Follow all procedures and policies listed the Fairview-University Medical Center Laboratory Safety Manual. Consider all specimens, control materials, and calibrator materials as potentially infectious.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

The NHANES glucose results are entered onto a spreadsheet provided electronically by Westat, Inc for NHANES.

A. To access the spreadsheet click on My Computer → Z drive → User → Dep Labs → Collab Studies → NHANES → Glucose 009 or Glucose 098.

B. Choose the files named with the corresponding box number.

C. Enter the analysis date, run number, the technologist's initials, glucose results, and comment code.

D. The results will be sent electronically by the contact person.

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

A. Samples are collected and processed in mobile examination centers according to NHANES protocols.
B. Specimens are packaged and shipped on cold packs or dry ice according to the established schedule.

C. Specimens are shipped via Federal Express for delivery directly to Collaborative Studies Clinical Laboratory.

D. Specimens are stored at -70°C until analyzed.

E. Acceptable specimen types include non-hemolyzed serum or plasma (EDTA, heparin or sodium fluoride). Patient should be fasting unless a glucose challenge test is being administered. Because red blood cells reduce serum glucose levels via glycolysis at a rate of seven per cent per hour, prompt separation of cells from serum is essential (<60 minutes following collection). Serum glucose levels are stable for eight hours at room temperature; three days at 4ºC.

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

Glucose testing is performed on the Roche/Hitachi 911 instrument (Roche/Hitachi 911 Analyzer. Roche Diagnostics, 9115 Hague Road, P.O. Box 50446, Indianapolis, IN 46250-0446).

B. Materials

1. Roche product #450058, glucose reagent kit:
   R1 buffer (10 x 95 mL). Tris buffer, magnesium sulfate, ATP, NADP. See insert for concentrations. R1 stable for 28 days refrigerated on the instrument

2. R2 reagent (10 x 18 mL). Hepes buffer, magnesium sulfate, hexokinase, G6PD. See insert for concentrations and activities. Storage and stability: R2 stable for 28 days refrigerated on the instrument

3. Purified water supply. The 911 requires a continuous supply of purified water. The system used by the 911 is serviced by U.S. Filter, Lowell, MA 01851. The water is filtered through two mixed bed resin tanks as well as an inline carbon filter and a 0.2 micron final filter.

4. Reaction cells. Part no. 7070670. Cells are manufactured in 20-cell segments. Six segments complete the rotor. Cells must be soaked overnight in two per cent hitergent prior to use. This removes interfering substances deposited during production.

5. Tractor-feed, 8.5 x 11 inch, printer paper. Various sources.

6. Printer cartridge. Part no. DEC LA 30RKA. Corporate Express, Arden Hills, MN 55112.

7. Macrosample cups (2 mL). Part no. 40904100.

8. Microsample cups (0.5 mL). Part no. 1406680.

C. Reagent Preparation

1. The reagents are instrument-ready. They do not require any preparation.

2. Hitergent. Part no. 409149 (1L), 1135329 (100mL). Non-ionic, bacteriostatic detergent. Automatically added to reaction bath (from 100 mL bottles onboard in both reagent rotors) to minimize bubble formation that could hinder photometric measurements. Manually combined with Cell Clean 90 (from 1L bottle) to clean reaction cells.

3. Cell Clean 90. Part no. 1224310 (2L). Sodium hydroxide-based detergent. Used to clean reaction cells between uses. Received in liquid form. Add 20 mL of Hitergent to the 2L bottle of Clean Cell 90. Mix thoroughly, and then transfer to holding tank located on a pullout rack in the left front of the instrument.

D. Standard (Calibrator) Preparation

Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The calibrator is stable until the expiration date on the bottle when stored at 4ºC. Lyophilized calibrator is prepared with 3.0 mL of diluent, provided with the kit. Volumetrically add the diluent, and then dissolve by gentle swirling within 30 minutes. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once).

Caution: This product is of human and animal origin. Handle as though capable of transmitting infectious disease.

E. Preparation Of Quality Control Material

Two levels of control are assayed each time the glucose method is performed. Consult quality control chart for current ranges and lots in use.

1. Low control pool – aliquots are stored frozen at -70°C. Thaw and mix well before use.

2. High control – PPU purchased lyophilized. Dilute with 3 ml of the provided diluent. The diluted control is stable for 3 days at 4°C.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

The 911 will automatically calibrate glucose when there is a reagent lot number change. There is no automatic time-dependent calibration. Monitor control values to determine stability of the current calibration.

A. Setup/Calibration

There is a variety of calibration models used on the 911. There are factored methods, blank calibrations, two-point calibrations and multi-point calibrations. The type of calibration is dictated by the Roche chemistry parameters for each method. The calibrator material may be a Roche product, an in-house preparation, or a product from another company. When the lot number of a calibrator changes, Roche provides a diskette to automatically update the setpoints on all affected methods. This update (and other parameter edits) may also be performed manually.

Frequency of calibration is dictated by automatic time-dependent re-calibration built into the chemistry parameter profile for each test, and by observing the quality control data. All methods do not have an automatic time-out calibration feature. Details for each assay may be found in specific procedures. Acceptable accuracy and precision limits are defined in each chemistry parameter file.

B. Loading Calibrators and Controls

Each calibrator and control has a defined location on the most inner circle of the sample rotor or, occasionally, in the middle circle (the outside circle of the sample rotor is for specimens). A complete posting of all of these assigned locations is available at the desk. The inside sample rotor is refrigerated, and is covered with a dome to ensure constant temperature throughout the day. A small label is attached at the specific positions for each of the calibrators and controls. This location is also indicated in the chemistry parameters for each method. See specific protocols for the appropriate calibrators and controls to be used for each test.

C. Requesting a Calibration: (Operator's Manual Section 2)

1. <Routine> job key.

2. <2> Calibration Test Selection, <Enter>

3. Mode field. Choose <2> Repeat Calibration, <Enter>.

4. Type field. Choose the appropriate calibration type for the method needing recalibration. All enzyme methods utilize a "Blank" calibration. Most of the other methods are "2-point". There are also several methods utilizing a "Full" calibration. The correct type of calibration for each method can be found in the specific test protocols located in the instrument's Parameters file. <Enter>.

5. Tests. Select tests to be calibrated. <Enter>.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Procedure

1. Instrument Set-up

The instrument should be placed in Sleep mode when not in use for long periods (e.g. overnight). This prolongs the lamp life and other essential components. The Sleep function allows for a preprogrammed wake up time, but if this function must be overridden, move the cursor to the on/off location, and turn off the Sleep function by pressing <2>, <enter>, <1>.

The 911 has scheduled daily, weekly and monthly maintenance. These tasks are listed in the check-off chart at the instrument, and described in detail in Chapter 3 of the Operator's Manual. Perform all scheduled maintenance before beginning testing.

2. Loading Reagents: (Operator's Manual Section 2)

There are two reagent carousels: R1 and R2. Like the QC rotor the two reagent carousel chambers are refrigerated, with a locking lid to ensure constant temperature. There are 33 positions in each rotor. All reagent containers have a barcoded ID on their side. This barcode contains lot number, test code, expiration date and available test count information. Most methods use two reagents (R1 and R2), though some use one or four. If a test volume greater than the capacity of one reagent container is anticipated, additional bottles may be placed on the instrument. The 911 will

automatically begin pipetting a new bottle once the previous bottle is empty. If the reagents are placed in incorrect rotors, or if the barcode is illegible, the 911 will not allow performance of the assay. Reagents may be placed in any position of the rotor since the barcode directs the 911 to the correct location for pipetting. The 911 reads the barcodes each time a lid is removed and replaced over the reagent chamber.

B. Quality Control Materials

Two levels of controls are assayed each day that a specific test is performed. Check current QC records for lot in use and acceptable values.

C. Operation

Loading Specimens: (Operator's Manual Section 2)

These instructions are provided under the assumption that all sample IDs and test codes will be manually entered by the 911 user. The instructions for specimen loading utilizing the bi-directional interface and barcode reader can be found in the separate 911 interface protocol.

1. Transfer the specimen from its original labeled container to a 911 sample cup.

2. Place the cup into the next available sample position in the outer sample ring.

3. With the original container still in hand, enter the desired demographics for the specimen:

   a. <Routine> job key.
   b. <3 Patient Test Selection> <Enter>
   c. On the Patient Test Selection screen, enter the three parameters defining the Sample Number.
      (1)Record number. The 911 counts each specimen as one record number. For the first specimen of the day, this field should read "1". As each specimen is loaded onto the instrument, this number indexes by one.
      (2)Disk number. Begin the day with rotor "0". As each set of 50 specimens is complete (50 is the capacity of each rotor), this number will automatically index by one. In order to keep the sampling system functioning past position 50, reset the disk number to "0" at record numbers 51, 101, 151, etc. If the disk number is allowed to index automatically, the 911 will stop sampling at cup 50. It will not re-start until the start button is pressed.
      (3)Position number. This is the position of the specimen on the rack.
   d. Enter the specimen ID number. <Enter>
   e. Select the tests desired on the specimen. <Enter>

4. Continue this process until 10-15 specimens have been ordered. Additional specimen loading can occur while the instrument is sampling.

5. If the reagent status is acceptable and the necessary calibrations have been ordered, select the <Routine> job key, option <4> Start Conditions.

6. In the Start Conditions screen, choose as the Start Sample Number the record number of the first specimen ordered in the most recent sequence. This number will index as the specimens are completed by the 911.

7. Move the cursor to the Repeat Calibration field. Select <1> ON, <Enter> if there is a calibration to be performed. This field will default to OFF if not changed.
8. The other options on this screen have uses described in Section 2.23 of the Operator's Manual. The primary field of interest is the Manual Masking field. Move the cursor to this field and select any test key that the operator would like temporarily disabled. This is usually done when a time-out calibration is scheduled to occur on a test that is not being performed at that time. This avoids the steps of loading the calibrators and controls that would be required for that process.

9. Press the Start key. The instrument will go through its homing checks, wash several reaction cells, and then begin delivering the calibrators, controls, specimens and reagents to the reaction cells.

10. After this process has begun, additional tests may be ordered by returning to the Patient Test Selection screen. Tests may be added at anytime, but once the 911 enters "Yellow Operation" mode, those tests ordered after that point will not be sampled in the current sequence. All sampling and reporting will need to be completed before the Start button is pressed again to finish the testing ordered after that point.

9. REPORTABLE RANGE OF RESULTS

The linear range of the method is 2 – 750 mg/dL.

If a manual dilution is required, dilute the specimen in normal saline and multiply by the dilution factor.

10. QUALITY CONTROL (QC) PROCEDURES

Two levels of control are assayed each time the glucose method is performed. Westgard rules are followed as outlined in the general laboratory Quality Control and Quality Assurance procedure. Controls are analyzed at the beginning of a run, periodically throughout, and at the end of a run.

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

If control values are out of the acceptable range, recalibration is required. Reanalyze any patient samples after recalibration.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

There are no significant interferences due to icterus, hemolysis, or lipemia.

13. REFERENCES RANGES (NORMAL VALUES)

Adults: 60-100 mg/dl (American Diabetes Association)

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Alert values for studies: <60 mg/dl and >200 mg/dl. Contact field center when these values occur.

Early reporting for NHANES: >125 mg/dl for fasting glucose or ≥140 mg/dl for non-fasting glucose. Notify the NHANES Medical Officer. The contact person will electronically send these results as soon as possible.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are stored at -70°C until analyzed. On the day of analysis, thaw the specimens. Mix thoroughly. Upon completion of analysis, refreeze at -70°C. Specimens are discarded after one year.

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

If the instrument is unable is perform the test, the specimens are stored at -70°C until testing is available.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS

The NHANES glucose results are entered onto a spreadsheet provided electronically by Westat, Inc for NHANES.

A. To access the spreadsheet click on My Computer → Z drive → User → Dep Labs → Collab Studies → NHANES → Glucose 009 or Glucose 098.

B. Choose the files named with the corresponding box number.

C. Enter the analysis date, run number, the technologist's initials, glucose results, and comment code.

D. The results will be sent electronically by the contact person.

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

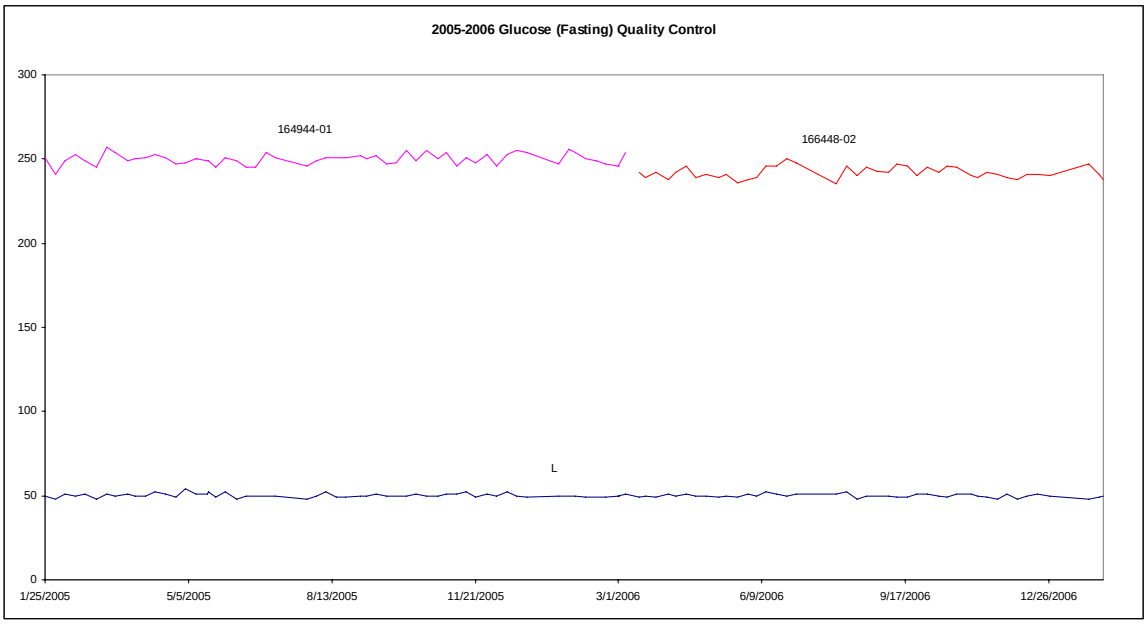
All shipments are recorded on the NHANES Shipping Log upon receipt. Actions taken during the course of analysis, result reporting, and specimen retention are also recorded on the log.

19. SUMMARY STATISTICS AND QC STATISTICS

A. Fasting glucose

Summary Statistics for Glucose (fasting) by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
L	100	1/25/2005	2/2/2007	50.1	1.11	2.2
164944-01	58	1/25/2005	3/6/2006	250.0	3.32	1.3
166448-02	42	3/15/2006	2/2/2007	241.9	3.49	1.4



REFERENCES

1. Roche/Hitachi System Application Sheet for glucose, 1997.
2. Package insert for C.F.A.S., December 2000.
3. Roche/Hitachi 911 System Operator's Manual, January 1997. Catalog #011002900.
4. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diab Care 2004: 27 (Supp 1): S5-S10.