Laboratory Procedure Manual

Analyte:	Oral Glucose Tolerance Test
Matrix:	Plasma
Method:	Hexokinase-mediated reaction Roche/Hitachi Modular P Chemistry Analyzer
as performed by:	*University of Minnesota Medical Center, Fairview* *Collaborative Studies Clinical Laboratory* *Minneapolis, Minnesota*
Contact:	Dr. Michael Steffes

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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 the items listed in the following table:

File Name	Variable Name	SAS Label (and SI units)
OGTT_E	LBXGLT	OGTT glucose(mg/dL)
	LBDGLTSI	OGTT Glucose (mmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Carbohydrates provide the body with glucose, the most important monosaccharide in the blood. Glucose is the substrate for many energy-producing cellular functions. Glucose degradation occurs through glycolysis. Measurement of glucose is useful in the diagnosis and monitoring of carbohydrate metabolism disorders. A partial list of these disorders would include diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia, and pancreatic islet cell carcinoma. The hexokinase method used on the Mod P is a recognized reference method.

Modular P application code: 767

Manual worksheet code: UVR

Principle:

In this enzymatic method glucose is converted to glucose-6-phosphate (G-6-P) by hexokinase in the presence of ATP, a phosphate donor. Glucose-6-phosphate dehydrogenase then converts the G-6-P to gluconate-6-P in the presence of NADP+. As the NADP+ is reduced to NADPH during this reaction, the resulting increase in absorbance at 340 nm (secondary wavelength = 700 nm) is measured. This is an endpoint reaction that is specific for glucose.

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. Use serum or plasma (EDTA, heparin, fluoride, or iodoacetate anticoagulants are acceptable) for the procedure. Other anticoagulants are unacceptable. Serum or plasma is stable for 8 hours at room temperature, three days at 4ºC, and longer at –70ºC.

E. Specimens must be centrifuged and separated within 30-45 minutes following collection. Red blood cells will metabolize glucose via glycolysis, and the measurable glucose will decrease if the cells are left in contact with the cells for a prolonged period of time. This decrease in concentration can be as much as 7 per cent per hour.

F. Plasma specimens that have been frozen (e.g. NHANES study) are especially prone to clot re-formation as they warm to room temperature. After thoroughly mixing these specimens, put a wooden stick into the plasma to remove any fibrin clots. These specimens are also prone to excessive precipitate formation. Centrifuge these specimens prior to analysis as well. Place the specimens on the Mod P as soon as possible after centrifugation.

G. Minimum volume: 100 uL (includes dead volume)

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, Materials:

1. Roche Modular P chemistry analyzer. Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250.
2. Cell Wash Solution II/Acid Wash. Roche product #4880307 (2L bottle). No preparation required. Solution of formic acid, citric acid and nikkol BT-9. Store at room temperature. Stable until expiration date on bottle. No stability time window after opening. This solution is automatically drawn by the Mod P while cleaning reaction cuvettes during analysis.
3. Cell Wash Solution I/NaOH-D. Roche product #1551540 (1800 mL bottle). No preparation required. Solution of sodium hydroxide (1N). Store at room temperature. Stable until expiration date on bottle. After opening a bottle it is stable for 14 days on the instrument. This solution is automatically drawn by the Mod P while cleaning reaction cuvettes during analysis.
4. Reaction cell cuvette segments. Roche product #714-0650 (Four sets of eight segments. Eight segments complete the entire rotor). Soak cuvettes overnight in a solution of 2% Hitergent before installing on the instrument. Perform cell wash and cell blank functions after installation. Change cuvettes quarterly.
5. Hitergent. Roche product #409149 (1L bottle). No preparation required. Solution of ethanolamine, hexahydro-1,3,5-tris (Betahydroxyethyl) triazine and nonidet P-40. Store at room temperature. Stable until expiration date on bottle. Hitergent is an on-board reagent automatically

drawn by the Mod P during the daily incubator bath exchange. Hitergent is transferred, as needed, from the 1L bottle to the 66 mL bottle located in position 2D3.

6. Sample cups (micro). Roche product #11406680001.
7. Sample cups (standard). Roche product #729177.

B. Reagents and Reagent Preparation:

1. Roche product #1876899, GLU reagent kit:
   a. R1 reagent (6 x 66 mL). TRIS buffer, pH 7.8, Mg^{2+}, ATP, NADP, preservative. See insert for concentrations. No preparation required. There are approximately 350 tests per bottle.
   b. R2 reagent (6 x 16 mL). HEPES buffer, pH 7.0, Mg^{2+}, hexokinase, glucose-6-phosphate dehydrogenase, preservative. See insert for concentrations. No preparation required. There are approximately 440 tests per bottle.
   c. Storage and stability. Keep reagents stored in refrigerator until use. R1 is stable for 28 days refrigerated on the analyzer. R2 is stable for 28 days refrigerated on the analyzer.
   d. Though the number of tests per bottle is slightly different, always change the reagents as a pair. When loading the reagents onto the Mod P, make sure R1 is placed in the R1 rotor, and R2 is placed in the R2 rotor. Remove any bubbles in the reagents prior to loading. Place the reagents in like-numbered locations in the two rotors. This makes it easier to track the chronology of the reagents on the instrument.
2. Milli-Q water. Milli-Q is the trade name of the water system purchase from the Millipore Corporation. Milli-Q is deionized water treated with activated carbon and deionization cartridges and filtered to remove microorganisms larger than 0.22 micrometers. This meets CAP class I water requirements.

C. Calibrator:

1. 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. The lyophilized calibrator is prepared with 3.0 mL of Milli-Q water. Volumetrically add the water, and then dissolve by gentle swirling within 30 minutes. Avoid formation of foam while mixing. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once).

2. The C.F.A.S. calibrator is traceable to reference material SRM 965 (IDMS). This is a reference material provided by the National Institute of Standards and Technology.

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

D. Controls:
   1. Two levels of control are assayed each time the glucose method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired.

2. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult quality control charts for current ranges and lots in use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration frequency:
1. The Mod P will automatically perform a two-point calibration when there is a reagent lot number change. No other auto-calibrations are defined for the glucose assay. The Mod P will not allow testing to proceed until a successful calibration has been completed.
2. Monitor control values to determine stability of the current calibration

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Instrument Setup:

1. Log into the Mod P using assigned username and password.
2. Reagents. All reagents used on the Mod P are stored in a refrigerated reagent compartment. Glucose is a two-reagent system. Reagent 1 must be placed in the outer (R1) rotor; reagent 2 must be placed in the inner (R2) rotor. All reagents have a unique barcoded identifier. Before starting the analysis sequence check the reagent status on the Mod P to confirm there is adequate reagent to complete the anticipated test volume for the day. Discard any bottles that have gone empty. Check the volume of the two wash reagents.
3. Maintenance. Complete the scheduled daily maintenance as described in the Mod P general operations protocol.
4. Order calibration, if indicated (see Mod P general operations protocol).
5. Order controls. If a calibration was requested, the controls should not be ordered until the calibration report has printed. If the controls are ordered and executed before the calibration prints out, the controls will be measured on the previously stored calibration line.

B. Procedure:

After calibration and controls have been measured and evaluated, the test specimens may be loaded onto the Mod P. An abbreviated description of the measurement procedure follows. A more thorough description may be found in the Mod P general operations protocol.

1. If specimens have been frozen, allow them to thaw completely, then mix well. Serum specimens should not require centrifugation unless they have large amounts of suspended material (see Specimen section above).
2. To order <u>non-barcoded</u> tests on the Mod P:
    a. <Workplace>
    b. <Test Selection>
    c. Enter specimen ID in the Sample ID field, then <Enter>
    d. Select test GLU by touching the screen or clicking on it with the mouse.
    e. <Barcode Read Error>
    f. Enter the rack number and rack position in the Rack No.-Pos. fields.
    g. <Add>
    h. <OK>
    i. <Save>

3. Note the order of positions 1-5 in the sample rack: position 1 is on the right and position 5 is on the left. Place the specimen in the rack so that ¼ to 1 inch of the vial is above the sample rack. This allows the Mod P to detect the presence of the vial in the rack. Orient the vial in the rack so that any barcodes are turned inward, and therefore unreadable. If the testing vials are to be re-capped, arrange the caps so they can be matched up following analysis.
4. To order barcoded tests on the Mod P: Follow instructions as in step 3 above, except that the barcode now must face outward so the Mod P can read it. The barcode must be oriented vertically. No test ordering is required on the instrument. In this case test ordering has occurred in Misys, and a label has been generated for that purpose, or the specimen has a non-Misys barcode label and a user-defined default battery has been installed on the Mod P. The latter is the case for analyzing NHANES specimens. Before starting the NHANES specimens check that the default panel (Glucose) is correctly defined: <Start>, <Default Profile>, add or subtract panel from the menu as needed.
5. After the specimens are in place, put the racks onto the loading platform. The racks will only load in one orientation, as the center track is offset. Do not prepare more than three racks at a time, as evaporation could occur while the instrument goes through the sampling process.
6. Close the cover on the loading platform.
7. On the Mod P computer terminal, press or click <Start>, then <Start> again.
8. Only calibration and control data automatically print out. Patient data hard copies must be requested in <Workplace>, <Data Review>. Highlight the desired records, then <Print>, and <Print> again.
9. Non-barcoded records must be manually entered into Misys, a designated spreadsheet, or website.
10. Barcoded records are accepted using the OEM program in Misys. The method code for the Mod P is UR9.

C. Instrument shutdown:

1. After the patient specimens and final controls have been evaluated and accepted, load the green rack (W999) and run it through the instrument. Place three standard sample cups in positions 1, 2 and 3. Fill cup 1 with 1N sodium hydroxide, fill cup 2 with 4N sodium hydroxide, and fill cup 3 with leftover serum. Place it onto the loading platform and press <Start>, and <Start> again. After 18 minutes, the Mod P comes to Stand-by status. If the green rack is not run, the Mod P will take at least one hour to come to Stand-by status.
2. After coming to Stand-by status the data from each day's run is downloaded from the Mod P computer to a diskette, then to the network folder. Consult the procedure describing this process for details.
3. Print all Mod P test results, and file in chronological order with the other daily printouts.
4. The Mod P is turned off each day after all work is complete. The steps are as follows:<Utility>, <Maintenance>, <Nightly Pipe>, <Select>, <Execute>. This shutdown process requires approximately five minutes. The instrument and its computer are automatically turned off. The reagent compartment remains refrigerated.
5. An automatic timer has been set so that the Mod P turns on each weekday morning at 0630, automatically performing an air purge, photometer check, and incubator bath exchange during the process. The automatic timer has been set so that the Mod P remains off during weekends.
6. Return all leftover controls and calibrators to the refrigerator at the end of the day.

9. REPORTABLE RANGE OF RESULTS

A. Expected Values:

1. Roche ranges:
   Plasma, adult: 70-115 mg/dL

2. Collaborative Studies Clinical Laboratory ranges:
   Misys test code GLUR:
   Serum, adult: 60-99 mg/dL

3. Linear range of the method: 0-750 mg/dL (serum). Specimens exceeding the high limit are automatically diluted (1:2) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.

4. Glucose results by this hexokinase method demonstrate an approximate 3 per cent positive bias when compared to those obtained with a glucose oxidase/oxygen electrode method.

5. Analytical Measurement Range: 0-750 mg/dL

6. Clinically Reportable Range: 2-2000 mg/dL

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

A. Interfering Substances and Conditions:

1. Bilirubin does not interfere up to an I index of 60.
2. Hemolysis does not interfere up to an H index of 1000.
3. Lipemia does not interfere up to an L index 1000.

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.

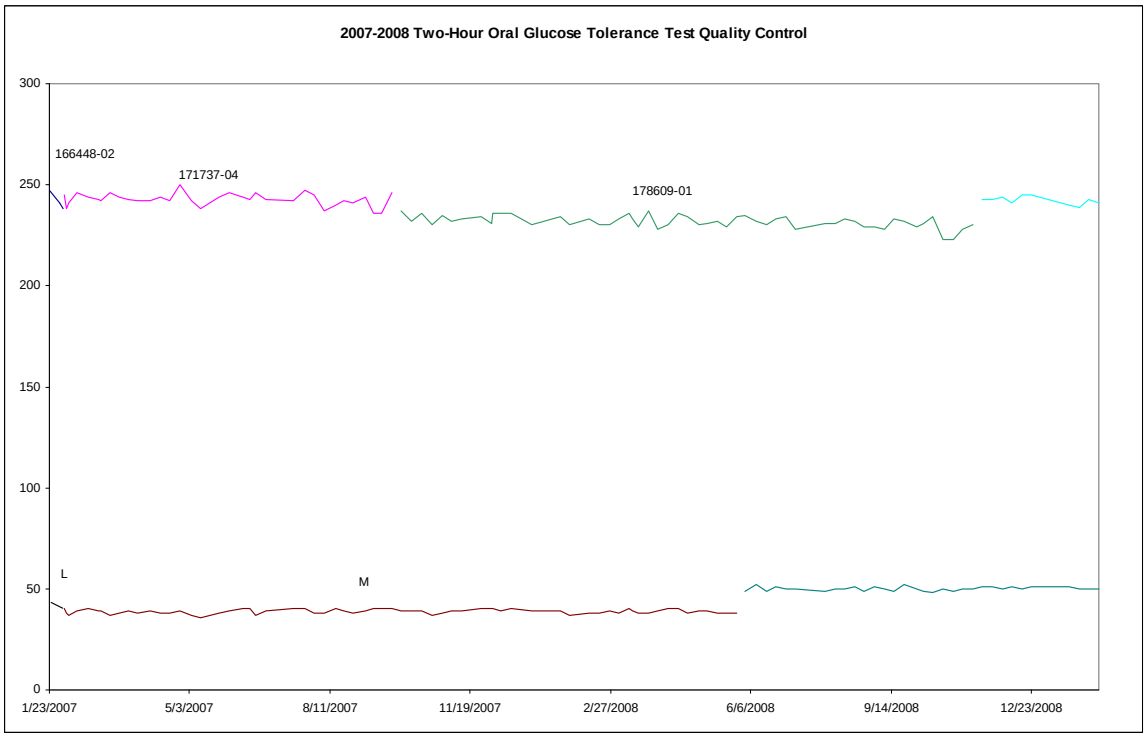
18. TRANSFER OF REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

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

Summary Statistics for Two-Hour Oral Glucose Tolerance Test by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
L	3	1/23/2007	2/2/2007	49.000	1.000	2.0
166448-02	3	1/23/2007	2/2/2007	242.000	4.583	1.9
M	67	2/3/2007	5/27/2008	38.731	0.994	2.6
171737-04	35	2/3/2007	9/24/2007	242.829	3.139	1.3
178609-01	54	10/1/2007	11/11/2008	231.759	3.083	1.3
N	32	6/2/2008	2/9/2009	50.094	0.928	1.9
180161-02	10	11/18/2008	2/9/2009	242.400	2.066	0.9



References:

1. Roche/Hitachi System Application Sheet for Glucose/HK, 2004.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.
4. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diab Care 2004: 27 (Supp 1): S5-S10.