

Laboratory Procedure Manual

Analyte: Fasting Glucose

Matrix: Plasma

Method: Roche Cobas C311 2017—Standard

As performed by: University of Missouri-Columbia

Contact: Dr. Randie Little

Important Information for Users

The University of Missouri 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)
GLU_I OGTT_I	LBXGLU	Fasting glucose(mg/dL)
	LBDGLUSI	Fasting glucose (mmol/L)
	LBXGLT	Two-hour glucose (OGTT) (mg/dL)
	LBDGLTSI	Two-hour glucose (OGTT) (mmol/L)

I. Purpose Statement

- a. This SOP is intended for the *in vitro* test for the quantitative determination of glucose in human serum and plasma. It is performed on the Roche Cobas C311 system in the Diabetes Diagnostic Laboratory.

II. Definitions

- a. Not $\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$ Applicable

III. Content

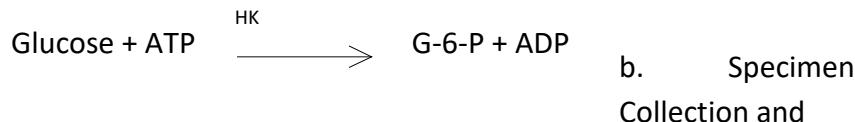
- a. Principle and Clinical Significance

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^{1,2,3,4}.

The cobas c311 performs a UV test to detect glucose in blood serum and plasma.

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.

The enzymatic reference method is performed with hexokinase.⁴ Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.



Handling

i. Patient Preparation

1. Patients should be fasting or undergoing an Oral Glucose Tolerance Test. Patient's status should be recorded when specimen is drawn.

ii. Specimen Type

1. Collect blood by venipuncture from individuals using an evacuated tube system
 - a. Plasma: Fluoride plasma for NHANES study testing (grey top tubes)
2. The minimum volume required for analysis directly from collection tube is 200 µL.
3. Specimens are delivered to the Diabetes Diagnostic Laboratory, Room M764 by FedEx. Specimens are received frozen on dry ice and are stored at -70°C. Each specimen must arrive in the laboratory labeled with two unique accession number generated by NHANES, and checked against the specimen manifest.

iii. Specimen Stability

- | | |
|---|---------------------------|
| 1. Stability in fluoride plasma: ⁵ | 3 days at 15-25 °C |
| | At least 3 months @ -20°C |
| | At least 2 years @-70°C |
2. Unacceptable specimen criteria
 - a. Clotted specimens.

- b. Unlabeled samples
 - c. Specimens not collected in sodium fluoride.
 - d. Whole blood specimens
 - e. Refer to limitations section for additional details
 - f. The below must be followed for unaccepted specimen collections:
 - i. Record all unacceptable samples on sample manifest
 - ii. Contact NHANES regarding unacceptable specimen
- iv. Handling Conditions
1. For specimen collection and preparation, only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable: Plasma: fluoride plasma for NHANES study testing. (Grey-top tubes)
 2. To minimize glycolysis, one should place the sample tube immediately in an ice-water slurry, and the plasma should be separated from the cells within 30 min. Blood for FPG analysis should be drawn in the morning after the individual has fasted overnight (at least 8 hours).
 3. If testing is not performed immediately, samples are to be maintained under frozen conditions (-70°C).
 4. For NHANES samples: Laboratory services are requested through the Westat system operations via an email notification containing a unique manifest list of the samples and sample analysis type (e.g. Glucose), which confirms that specimens have been shipped to DDL. Transport under frozen conditions.
 5. Each Manifest Form should include and verified against each sample received:
 - a. Patient Sample ID #
 - b. Test Name
 - c. Date Collected
 - d. Shipment ID #
 - e. Shipment Date
 - f. Lab Name
 - g. Lab ID
 - h. Survey Year
 6. Once received and prepared for analysis, specimens are to be immediately returned to -70°C storage. When ready to be analyzed, specimens are to be thawed at room temperature.

Following testing, specimens are to be returned to -70°C for long-term storage.

c. Equipment and Materials

i. Equipment

1. Roche Cobas C311 analyzer consists of the following main components:
 - a. Analyzer Unit
 - i. Sample disk: 110 position disk for loading of up to 108 samples
 - ii. Sample detectors and barcode readers: on inner and outer rings
 - iii. Sample Pipetting System: clot detecting by pressure measurements, liquid level detection by capacitance, and abnormal descent detection.
 1. Metal shield pipe
 2. Rinse station
 3. Drying cylinder
 4. Basic (sample cleaner 1) and acid wash (sample cleaner 2) solutions for special probe washes and maintenance functions.
 - iv. Reagent Compartment
 - v. Reagent Pipetting System
 - vi. Reaction Disk Area
 - vii. ISE System (not in use)
 - viii. Water supply tank
 - ix. Vacuum System
 - x. Sound Volume Knob
 - xi. Cell Wash Solutions
 - xii. Waste Container
 - xiii. Inlet Water Line
 - xiv. Dilute Waste Line
 - b. Control Unit
 - i. Computer
 1. Hard Disk Drive
 2. 3-1/2 in floppy disk drive and a DVD-RW rewrite device
 - ii. Keyboard
 - iii. Mouse
 - iv. Color Monitor: touch screen
 - v. Printer

ii. Materials

1. Reagents and other items
 - a. R1: MES buffer: 5.0 mmol/L, pH 6.0; Mg²⁺: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative
 - b. R2: HEPES buffer: 200 mmol/L, pH 8.0; Mg²⁺: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G-6-PDH (E. coli): ≥ 300 µkat/L; preservative
 - c. Sample vials and false bottom tubes
 - d. Printer paper.
 - e. Toner cartridge.
 - f. Refer to MSDS located in the Operator's Manual for reagent description and composition
2. Other materials
 - a. Powder free hypoallergenic latex examination gloves.
 - b. Biohazardous waste storage bags and boxes (Jefferson Smufit Corporation, Highland, IL).
 - c. Viro Research™ Envirocide Disinfectant Decontaminant Cleaner (Fisher Scientific, St. Louis, MO)
 - d. Transfer pipettes (Fisher Scientific, St. Louis, MO)
 - e. Single fold paper towels (Ft. Howard Corp. Co, Green Bay, WI).
 - f. Kim Wipe lintless tissues (Kimberly Clark Corp., Roswell, GA).
 - g. Low and high in-house plasma controls (low and high, respectively).
 - h. Bio-Rad controls
 - i. Volumetric pipettes for measuring different volumes.

iii. Equipment Maintenance

1. Roche Cobas c311– Routine maintenance
 - a. Perform daily maintenance as outlined in Cobas c311 manual every time machine is used
 - b. Perform weekly maintenance as outlined in Cobas c311 manual once a week (generally whenever machine is used)
 - c. Perform monthly, quarterly and bi-annually maintenance when assigned
2. Record all daily, weekly, monthly, and periodical maintenance in the “C311 Maintenance” binder. This paperwork is approved by the supervisor monthly and filed in “C311 Maintenance” binder.
3. Roche – Periodic/Preventative maintenance – Preventative maintenance inspections will be done at least yearly.
4. Pipettes are to be calibrated once a year.

5. Temperatures for the refrigerators and freezers where specimens, controls and calibrators are stored are to be recorded. Any readings that fall outside acceptable ranges are to be reported to the supervisor for corrective action. These areas are monitored by 7 day 24 hour temperature recorders for minimum and maximum temperatures and are checked daily (working day) by a technician to make sure the temperatures are within acceptable ranges. The charts are to be checked and initialed by the supervisor at least weekly.

iv. Storage Requirements – Reagent Use and Storage:

1. *GLUC3*
 - a. Shelf life at 2-8 °C: see expiration date on cobas c pack label
 - b. On-board, in use and refrigerated on the analyzer: 8 weeks
2. *Diluent NaCl 9 %*
 - a. Shelf life at 2-8 °C: see expiration date on cobas c pack label
 - b. On-board, in use and refrigerated on the analyzer: 12 weeks
3. All reagents are ready for use; to load packs onto machine, follow instructions in Cobas c311 analyzer Basic Operator Training Guide.

v. Reagent Labeling: Reagents, calibrators, controls, and solutions should be traceably identified to indicate the following:

1. Content and quantity, concentration or titer
2. Storage requirements.
3. Date
4. The below should be followed for reagents used daily
 - a. Preparation date or opened and the identity of the preparer;
 - b. Tech's initials
5. When new reagents are received they must be initialed and dated by the technician who receives them. When the reagents are opened they are initialed and dated with open date by the technician.
 - a. Regent packs loaded onto the machine are logged by the machine with the load date and will flag when expired.

d. Calibration:

- i. Preparation and Stability

1. Material: C.f.a.s (Calibrator for automated systems) manufactured by Roche Cobas. Human serum with chemical additives and material of biological origin as specified on package insert.
 2. Calibrator values are given in the electronically available value sheets, organized by lot. Determinations were performed under strictly standardized conditions on multiple Roche analyzers using Roche system reagents and C.f.a.s. master calibrator or reference materials.
 - a. Barcodes are given with each package of calibrator. They may be used to scan the calibrator lot into the c311
 3. Carefully open a bottle avoiding the loss of lyophilizate. Using a 3 mL volumetric pipette, add in exactly 3.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional swirling within 30 minutes. Avoid formation of foam.
 4. Storage and Stability
 - a. Store unopened C.f.a.s at 2-8°C
 - i. Stability of lyophilized calibrator at 2-8°C: see expiration date
 - b. Stability of glucose in the reconstituted calibrator
 - i. 15-25 °C: 8 hours
 - ii. 2-8 °C: 2 days
 - iii. (-15)—(-25 °C) : 4 weeks (when frozen once)
- ii. Frequency of Calibration:
1. Calibration verification is performed when a system is first placed in service and at least every six months (or as specified by the manufacturer) thereafter for all quantitative tests. Recalibration is required (regardless of the length of time since last performed) immediately if any of the following occurs:
 - a. A change of reagent lots for chemically or physically active or critical components, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client test results or the range used to report patient/client test data
 - b. If QC materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem
 - c. After major maintenance or service. The Laboratory Director must determine what constitutes major maintenance or service.

- d. When recommended by the manufacturer.
 - iii. Calibration Procedure: To calibrate the c311 for glucose
 - 1. Calibrators: S1: H₂O
 - S2: C.f.a.s
 - 2. Calibration Mode: Linear 2-point calibration
 - 3. Choose the "Calibration" tab
 - 4. Select the glucose test
 - 5. Select "2 point" under the method window
 - 6. Insert calibrators and controls into the correct vial positions.
These positions can be found in the Calibration load list report and QC load list report
 - 7. Select "Save"
 - 8. Select the "Start" button
 - 9. Traceability: This method has been standardized against ID/MS.
 - 10. Calibration Acceptability 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 above do not result in correction of the "out-of-control" values for QC materials, troubleshoot the instruments and reagents until the system is back "in control"
 - d. Recalibrate the system using a new vial of glucose standard.
 - e. Reanalyze the calibrator, controls, and specimens.
Specimens are stable at 4-8 °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.
 - f. Refer to Operator's Manual for additional troubleshooting advice.
 - g. If the calibration is still unsuccessful speak with your supervisor for further help. Do not perform glucose analysis until the system is declared "in-control" again.
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- e. Quality Control:
 - i. 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 difference between duplicates is greater than 10%, the specimen is reanalyzed. Batch QC specimens are placed in the rack at the end of the entire run. The following criteria are for “batch QC.”

ii. Control Materials and Stability:

1. Lyphocheck Assayed Chemistry Control Levels 1 and 2
 - a. Prepared from human serum with added chemicals, purified biochemical material (tissue extracts of human and animal origin), therapeutic drugs, stabilizers and preservatives. This product is provided in lyophilized form for increased stability
 - b. Stability
 - i. Store unopened controls at 2-8°C
 - ii. Stability of lyophilized calibrator at 2-8°C: see expiration date
 - iii. Stability of Glucose in reconstituted controls
 1. 2-8 °C: 7 days
 2. Frozen : 30 days (when frozen once)

2. In-house controls IHL and IHH

- a. Controls were prepared by collecting 450 mL (one unit) of whole blood from two diabetic (IHH) and two non-diabetic (IHL) subjects. The blood was collected in blood bags without an anticoagulant. The serum was allowed to clot for 1 hour and then separated from the red blood cells by centrifugation in a refrigerated centrifuge (4°C) for 25 min at 1500 g. The serum was then removed from the red blood cells, aliquoted in 0.5-mL portions and stored at -70 °C or colder in Nalgene cryogenic vials.
- b. If the stock of these controls becomes low, another batch is 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.
- c. Stability
 - i. In liquid nitrogen: 5 years
 - ii. At -70 °C: 1 year
 - iii. 2-8 °C: 72 hours
 - iv. 20-25 °C: 24 hours

- v. Current stability studies can be found in the Quality Control Binder located in room M771.
- iii. Preparation for analysis
 1. For each assay a single use aliquot is prepared in the same manner as an unknown specimen as described in Procedure.
 2. Bio-Rad Controls: Using a volumetric pipet or equivalent, reconstitute each vial with 5 mL of distilled or deionized water. Replace the stopper and allow this product to stand for approximately 20 minutes swirling occasionally. Before sampling, gently swirl the vial several times to ensure homogeneity. After each use, promptly replace the stopper and return to the appropriate storage condition.
 3. In-House Controls: One vial of each control is thawed and used in each assay. Reconstitution is not required for these controls.
- iv. Frequency for control materials: All four batch QC controls are analyzed at the beginning and end of each assay.
- v. Mean and Ranges:
 1. Daily means and ranges of the controls are calculated from at least 20 inter-assay 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.
 - a. The means and ranges for the current controls are posted near the c311 instrument
 - b. Current control value assignments and limits can be found in the Quality Control Binder located in room M771.
 2. 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.
 3. Two types of QC charts are used in assessing the quality of an assay.
 - a. 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 or more characteristic runs. The

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

- i. The mean for one control from a single run falls outside the 99% confidence limits.
 - ii. The means for two controls from a single run fall outside the 95% confidence limits.
 - iii. 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.
- b. 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 or more characteristic runs. The QC guidelines for DDL declare a system as "out-of-control" if any of the following events occur:
- i. The daily range for one control exceeds the 99% confidence limit.
 - ii. The daily ranges for two controls exceed the 95% confidence limits.
 - iii. The daily ranges for one control from eight successive runs (excluding the runs in which the mean is within ± 1 SD or bias range) are all above the mean line (trend rule).
- c. 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.
- vi. Remedial Action if QC Systems Fail to Meet Acceptable Criteria

1. When QC results fail to meet the acceptable criteria, check the sample cup containing the QC specimen for bubbles and reanalyze the QC specimen.
2. If the QC results meet the acceptable criteria, accept the run and report the results.
3. If steps above do not result in correction of the "out-of-control" values for QC materials, troubleshoot the instruments and reagents until the system is back "in control"
4. Recalibrate the system using a new vial of glucose standard.
5. Reanalyze the calibrator, controls, and specimens. Specimens are stable at 4-8 °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.
6. 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.

f. Procedure – Stepwise:

- i. Special safety precautions:
 1. Gloves and lab coat are required for handling all human blood specimens.
 2. All plastic tips, sample vials, gloves, etc. that contact blood are considered contaminated and are to be placed in an approved waste container.
 3. Work surfaces are protected by absorbent pads. The pads are discarded into biohazardous waste container weekly or whenever blood contamination occurs. All work surfaces are wiped down with Envirocide™ weekly.
- ii. Initial processing of specimens - Clinical trial specimens are checked against the clinical trial data sheet to insure that the patient information matches. The specimens are placed at -70°C until ready to be tested. Barcodes are printed by the DDL that correspond with the NHANES accession numbers on the samples. False bottom tubes are labeled with these barcodes.
- iii. To run the Roche Cobas c311:
 1. Before turning on the analyzer, perform the maintenance checks as outlined on the cobas c311 analyzer Maintenance Log under the "Check" and "Hands-on" sections.

2. All healthcare personnel shall routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of any patient is anticipated. All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear appropriate Personal Protective Equipment (PPE), including facial protection, gloves, and protective clothing. Dispose of all biological samples and diluted specimens in a biohazard waste container at the end of analysis. Dispose of all liquid hazardous waste in properly labeled hazardous waste container.
3. Turn on the power to the analyzer and control unit. The Analytical Unit **must** be turned on before the control unit.
4. Log-in to the control unit using name: adm and password: adm
5. The system will perform the “Power On Pipe” automatically.
6. Ensure all “Push-button” maintenance functions were performed by checking the utility screen.
7. Perform any maintenance functions that have expired
8. Put machine into maintenance mode by flipping switch on the analyzer. Perform any necessary maintenance outlined in the Maintenance Log that has not already been done.
9. Return machine to operation mode
10. Go to “System Overview” screen. Ensure that temperature of the incubator is within $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ by clicking “AU” button and observing the core temperature.
11. Check the “Work Flow Guide” area at the top of the screen. If any of the first five buttons is highlighted, perform that action (Daily Maintenance, Sample Data Clear, Regent Preparing, Calibration and QC Select, Parameter Download). These actions should be automatically completed by the analyzer.
12. Calibrate if necessary following the calibration procedures.
13. Enter controls to run as samples
 - a. Go to Utility menu
 - b. Click on sample information
 - c. Type in control name
 - d. Hit enter
 - e. Select “Gluc” test button
 - f. Select “Save” test button
 - g. Enter “Barcode Read Error” mode
 - h. Enter name of control and position on machine
 - i. Repeat for all 8 controls (beginning and end)

14. Enter samples and tests to run
 - a. Go to Utility menu
 - b. Click on sample information
 - c. Scan barcode for sample
 - d. Hit enter
 - e. Select “Gluc” test button
 - f. Select “Save” test button
 - g. Repeat for all samples
15. To run samples, choose “Start” button at lower right of the screen
16. Hit “Start” when prompted
17. To manually shut down the instrument, choose the “Deactivate system” option.
18. Preliminaries:
 - a. All reagents should be at room temperature before assay.
 - b. Allow frozen reference standards, QC specimens, and any frozen blood samples to thaw. Mix all samples at least ten minutes before putting on the machine
19. Sample preparation: Using printed labels, label the appropriate sample vials with the corresponding sample identification, and place the whole, uncapped original tube into the sample vials.
20. After run is completed, print hard copy of the results and proceed to reporting results.

iv. Calculations:

1. Roche **cobas c** systems automatically calculate the analyte concentration of each sample.
 - a. Conversion factors: $\text{mmol/L} \times 18.02 = \text{mg/dL}$
 $\text{mmol/L} \times 0.1802 = \text{g/L}$
 $\text{mg/dL} \times 0.0555 = \text{mmol/L}$

v. Reporting results:

1. 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 Glucose Daily Diary Log database located on the network drive. The calibrator value is 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. The clinical trial sample values are automatically uploaded into the computer database in mg/dL units. Data check sheets are printed.
3. Any comments associated with the specimen are entered in the comment field. 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 otherwise unacceptable, the result field is left blank or a -1 is entered and an appropriate comment is entered in the assay comment field.
4. Recorded results are then to be checked against the original printout by the supervisor or, in the event the supervisor is not present, the designated senior technician. Record the date checked and initials on the c311 Diary Sheet. A copy of the data check sheet is kept in the appropriate data book at the Diabetes Diagnostic Laboratory at the University of Missouri.

g. Reference Ranges:

- i. ADA reference ranges for fasting individuals³
 1. Normal: < 100 mg/dL
 2. Pre-diabetic (impaired fasting glucose): 100-125 mg/dL
 3. Diabetic: ≥ 126 mg/dL
- ii. ADA reference ranges for 2-hour postprandial individuals³
 1. Normal: < 140 mg/dL
 2. Pre-diabetic (impaired glucose tolerance): 140-199 mg/dL
 3. Diabetic: ≥ 200 mg/dL

h. Procedures for Abnormal Results:

- i. Critical Value Adults and Children: < 40mg/dL or > 400mg/dL
- ii. Critical Value Infants < 1 month : < 40mg/dL or > 300 mg/dL
- iii. **Critical results must be repeated and verified.**
 1. Glucose results do not have STAT turnaround. We typically receive these samples at least 24 hours after collection. Therefore there is no “panic situation”.

i. Reporting Format:

- i. Results are expressed on the report as mg/dL
- ii. Measuring range for Plasma: 2-600 mg/dL without dilution
 1. 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. Lower detection limit: 2 mg/dL

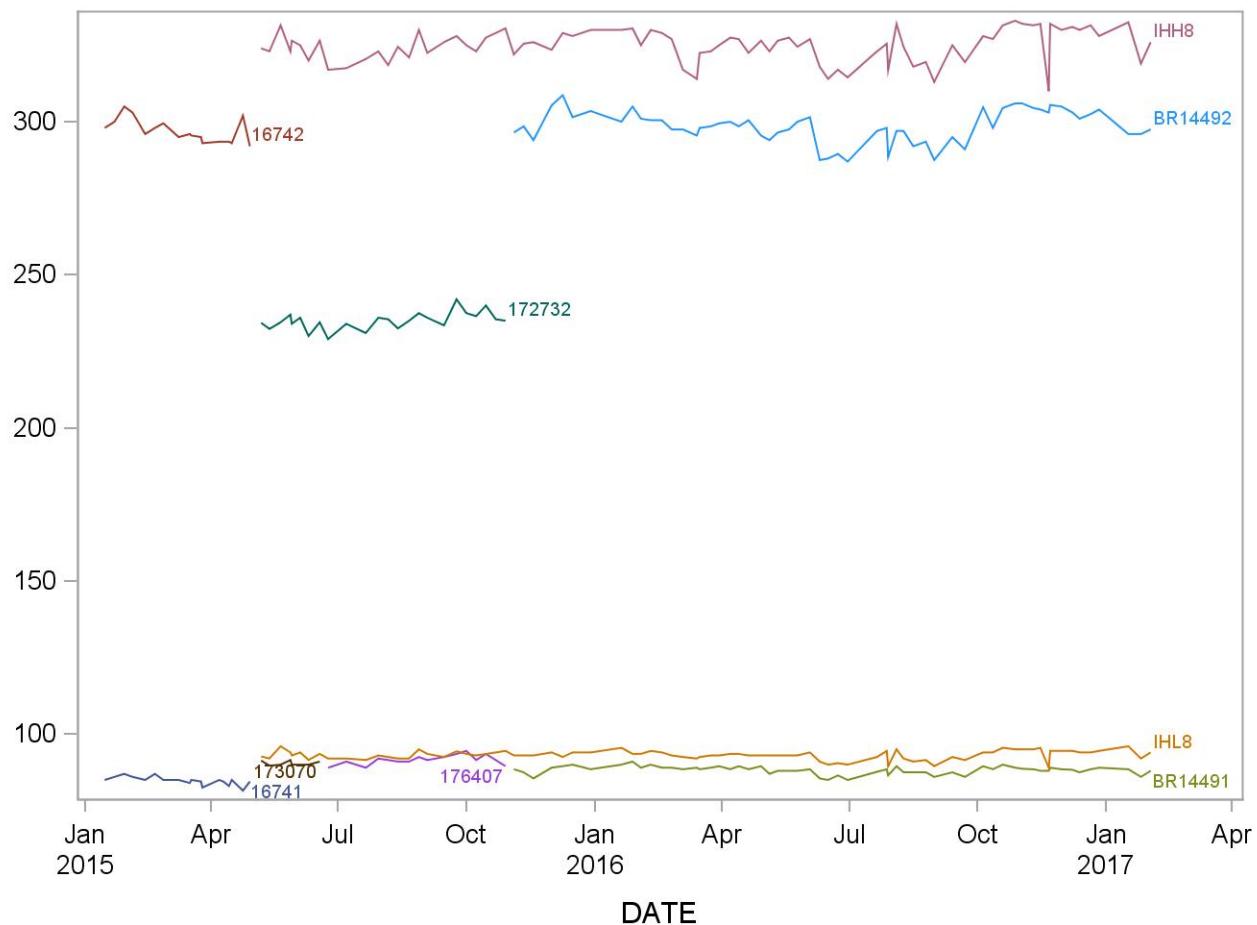
- a. The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD).
- iii. Linearity data is found in the c311 Maintenance Binder under the Linearity tab. Linearity studies are performed at least twice per year.
- j. Supervisor Responsibility:
 - i. The supervisor ensures quality control passes within the acceptable ranges prior to releasing patient results.
- k. Limitations of the Procedure/ Interfering Substances:
 - i. Criterion: Recovery within $\pm 10\%$ of initial value at a glucose concentration of 70.3 mg/dL.
 - ii. *Serum/plasma*
 - 1. Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL.)
 - 2. Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL).
 - 3. Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
 - 4. Drugs: No interference was found at therapeutic concentrations using common drug panels.^{7,8}
 - 5. In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
- iii. Actions Required
 - 1. Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
 - 2. Where required a special wash or carry over evasion programming must be implemented prior to reporting results with this test.

IV. SUMMARY STATISTICS AND QC STATISTICS

See next page

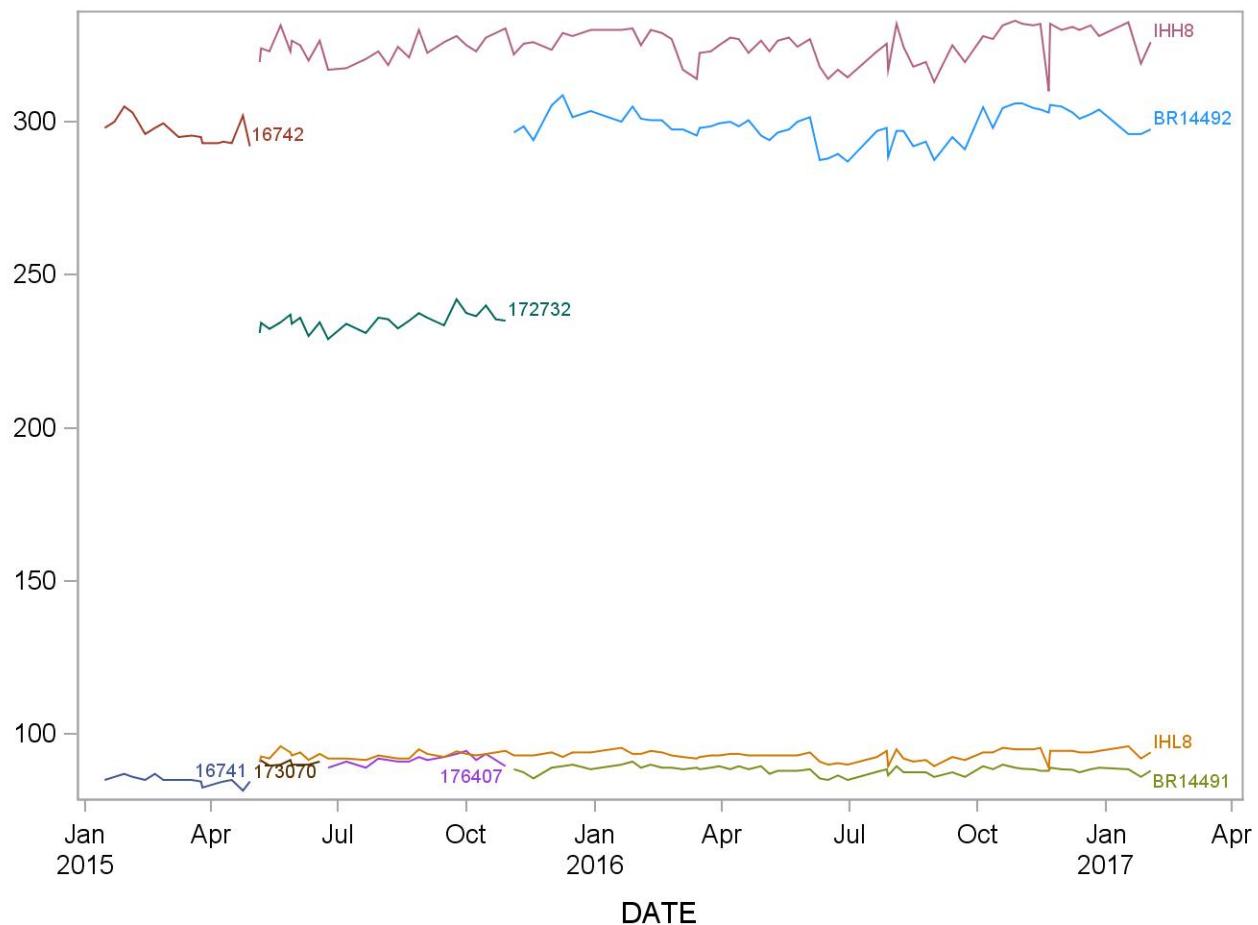
2015-2016 Summary Statistics and QC Chart for Fasting Glucose (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
16742	18	15JAN15	29APR15	296.8	3.8	1.3
16741	18	15JAN15	29APR15	84.8	1.4	1.6
IHH8	82	07MAY15	02FEB17	324.8	5.3	1.6
172732	24	07MAY15	29OCT15	235.0	2.9	1.2
IHL8	82	07MAY15	02FEB17	93.1	1.5	1.6
173070	8	07MAY15	18JUN15	90.4	0.7	0.8
176407	16	24JUN15	29OCT15	91.6	1.6	1.7
BR14492	58	04NOV15	02FEB17	298.5	5.3	1.8
BR14491	58	04NOV15	02FEB17	88.2	1.3	1.5



2015-2016 Summary Statistics and QC Chart for Two Hour Glucose (OGTT) (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
16742	16	15JAN15	29APR15	296.9688	3.9894	1.3
16741	16	15JAN15	29APR15	84.8438	1.4109	1.7
IHH8	83	06MAY15	02FEB17	324.7831	5.2737	1.6
172732	25	06MAY15	29OCT15	234.8067	2.9359	1.3
IHL8	83	06MAY15	02FEB17	93.1084	1.4976	1.6
173070	9	06MAY15	18JUN15	90.5000	0.6972	0.8
176407	16	24JUN15	29OCT15	91.5625	1.5586	1.7
BR14492	58	04NOV15	02FEB17	298.5388	5.2833	1.8
BR14491	58	04NOV15	02FEB17	88.2098	1.3137	1.5



V. References, Regulatory References, Related Documents, or Links

1. Sacks DB. Carbohydrates. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 4th ed. Philadelphia: WB Saunders 1996:351-374.
2. Knudson PE, Weinstock RS. Carbohydrates. In: Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. 20th ed. Philadelphia: WB Saunders 2001:211-223.
3. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1999:750-785.
4. Kunst A, Draeger B, Ziegenhorn J. In: Bergmeyer. Methods of Enzymatic Analysis, 3rd ed. Volume VI, Metabolites 1: Carbohydrates. 1984:163-172.