Title: Diabetes Diagnostic Laboratory - Glucose Testing using the Roche Cobas c311

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I. Purpose Statement

a. This SOP is intended for the *in vitro* test for the quantitative determination of glucose in human plasma. It is performed on the Roche Cobas c311 system in the Diabetes Diagnostic Laboratory.

II. Definitions

a. Not 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.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^{+}$$

b. Specimen Collection and 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. Only plasma specimens are acceptable.
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 AND
    ii. Contact NHANES ASAP 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, plasma 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 specimens are received and verified the corresponding file is imported electronically into the SQL server database via secure

transfer. Refer to DDL's NHANES study sample handling and reporting procedure for additional details.

7. After being verified specimens are to be immediately returned to -70°C storage. When ready to be analyzed, specimens are to be thawed at room temperature (~ 35 min). Following testing, specimens are to be returned to -70°C for long-term storage.
8. Refer to the Diabetes Diagnostic Laboratory NHANES study sample handling and reporting for additional details. https://muhealth.policytech.com/dotNet/documents/?docid=29520

c. Equipment and Materials
    i. Equipment
        1. Roche Cobas C311 analyzer consists of the following main components: Refer to the Operator's Manual attached in this procedure for **START UP** (Chapter 3), **OPERATION** (Chapter 2) and **SHUT DOWN** (Chapter 8) Procedures.
            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; Mg^{2+}: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative
   b. R2: HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G-6-PDH (E. coli): ≥ 300 µkat/L; preservative
   c. Glucose HK (GLUC3 REF 04404483).
   d. Diluent NaCl 9 % (REF 04489357).
   e. Calibrator (REF 10759350).
   f. Sample vials and false bottom tubes.
   g. Printer paper.
   h. Toner cartridge.
   i. 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 – Preventative maintenance is performed every 6 months.
    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 or delegate 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 or delegate 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. 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. Reagent 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 lyophilized material. Using a 3 mL volumetric pipette or equivalent, 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^{o}C
        i. Stability of lyophilized calibrator at 2-8^{o}C: see expiration date
    b. Stability of glucose in the reconstituted calibrator
        i. 15-25 ^{o}C: 8 hours
        ii. 2-8 ^{o}C: 2 days
        iii. (-15)—(-25 ^{o}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.

iii. Calibration Procedure: To calibrate the c311 for glucose
1. Calibrators: S1: diH_20
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 guidance (attached in this procedure) and the Appendix section for c311 Quick Reference guide.

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.

iv. Calibration Verification – The LN2 survey is purchased through CAP. The LN2 Calibration Verification/Linearity survey does not span the AMR for glucose. Therefore to verify the lower end of the AMR a serial dilution using the lowest LN2 sample is performed using a three twofold dilutions (e.g. 1/2, 1/4, 1/8). Aliquots are assayed in duplicate and the results regressed in EP evaluator against a Total Error of 12% (per CAP stated evaluation report criteria for linearity assessment).

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 % of the original value, 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. Lyphochek 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^{o}C
            ii. Stability of lyophilized control at 2-8^{o}C: see expiration date
            iii. Stability of Glucose in reconstituted controls
               1. 2-8 ^{o}C: 7 days
               2. -70 ^{o}C : up to 3 months

      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^{o}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. At -70 °C: 13 years
    ii. 2-8 °C: 72 hours
    iii. 20-25 °C: 24 hours
    iv. See glucose validation documents for In-house control & manufacturer control stability studies.

iii. Preparation for analysis
1. Bring frozen samples and controls to room temperature prior to analysis.
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.
4. Controls should be discarded after daily use.

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 within1 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 2-8 °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.

vii. To further validate the accuracy (and for troubleshooting purposes) of glucose measurements on the C311, Reference Material for Clinical Chemistry Standards (ReCCS) samples are analyzed every 6 months.

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^{o}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°C ± 0.1°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: mmol/L x 18.02 = mg/dL
           mmol/L x 0.1802 = g/L
           mg/dL x 0.0555 = 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.

2. 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.
3. After analysis the results, date analyzed and tech initials are imported from the instrument into the SQL server database via secure transfer.
4. Data check sheets are printed out and checked against the instrument printouts by the supervisor or delegate (signed by a supervisor or delegate).
5. After results are cleared by the supervisor or delegate a result file in the specified format is exported and uploaded to Westat via secure transfer.

g. Reference Ranges:
    i. ADA reference ranges for fasting individuals[3]
        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 individuals[3]
        1. Normal: < 140 mg/dL
        2. Pre-diabetic (impaired glucose tolerance): 140-199 mg/dL
        3. Diabetic: ≥ 200 mg/dL

h. Carryover Studies- Performed following manufacturer's experimental design and guidelines;
        1. Prepare two specimens, one with a very high %HbA1c (≥ 16 %, preferred HbA1c; ≥ 18.5%) and one with a very low %HbA1c value (≤ 4.8%, preferred HbA1c; ≤ 3.8%).
        2. Aliquot these specimens: 11 with low % HbA1c concentration and 10 with high %HbA1c concentration.
        3. While assaying these 21 samples, other specimens or tests should be assayed on the instrument and the samples should be assayed

in the following carryover order, **otherwise the experiment is invalid:**

3 L (low) / 2 H (high)/ 1 L /2 H/4 L/2 H /1 L/2 H/1 L/2 H/1 L

Note: Ensure at least 1 set of QC is analyzed within the run to ensure validity of results.

4. After analyzing the low and high specimens in the above order, enter the results in EP Evaluator using the carryover program. Refer to the EP Evaluator carryover report interpretation guide for additional details. [o, p]
5. The carryover test passes if the results of the High-low sequences are statistically identical to the results of the low-low sequences (three times the SD of the low-low result – the SD that would be expected if no high results were measured). The results of the low-level sample should not be affected by the high-level sample.
6. Expected Performance:
   i. No more than 0.2 % HbA1c carryover from H-L into L-L sample with samples beyond the selected low to high % HbA1c range is considered acceptable.
7. Carryover studies should be performed, as applicable, as part the initial evaluation of an instrument. Carryover studies should be repeated after major instrument maintenance (as specified by manufacturer) or repair of the pipetting assembly.

j. Procedures for Abnormal Results:
   i. Critical Value Adults and Children: < 40mg/dL or > 400mg/dL
   ii. 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”.

k. Reporting Format:
   i. Results are expressed on the report as mg/dL
   ii. Measuring range for Plasma: 2-728 mg/dL without dilution. Glucose results that exceed 728 mg/dL are reported as “> 728 mg/dL.”
   iii. Lower detection limit: 2 mg/dL
      a. The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero.

iv. Linearity data are found in the c311 Maintenance Binder under the Linearity tab. Linearity and AMR verification studies are performed at least twice per year refer to DDL's QM Program for additional details.

l. Supervisor Responsibility:
   i. The supervisor or delegate ensures quality control passes within the acceptable ranges prior to releasing patient results.

m. Limitations of the Procedure/ Interfering Substances:
   i. Criterion: Recovery within ± 10 % of initial value at a glucose concentration of 70.3 mg/dL.
   ii. *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 NaOH/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. Attachments:

a. C311 Operator's Manual

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.
5. Roche Diagnostic Technical support: 1-800-428-2336

VI. Appendix

cobas c 311 analyzer Quick Reference Card

Calibration

Before performing any of the functions on this card

1. Log on with a supervisor or administrator level password.
2. Make sure that the system is in Stand By.

Downloading new calibrator lot numbers via cobas® link

1. Click on the **Calibration** menu button, then the **Install** tab.
2. Click on the **Download** button located in the bottom right of the window.
3. Click on the **Lot Number** radio button. Type in the first six digits of the lot number followed by two zeros (e.g., lot number 181938-01 is entered as 18193800).
4. Click on the **Release Date** radio button.
5. Click on the **Search** button.
6. Click on the selection box to mark it for the most recent version.

   Note: *If Release Date was selected prior to the Search button, the most recent version should be the top item listed.*
7. Click on the **Download** button.
8. The File Transfer dialog box opens. Click on **OK** to proceed with download.
9. After the Confirmation window opens, click on **OK** to confirm.

Manually installing new calibrator lot numbers

1. Click on the **Calibration** menu button, then the **Install** tab.
2. Click on the calibrator to be modified.
3. Click on the **Add** button located on the bottom left corner of the window.
4. Click on the **Lot No.** field and enter the new lot number.
5. Click on the **Expiration Date** field. Enter the year of expiration in the left field. Enter the month of expiration in the right field, then click **OK**.
6. Click on the **Yes** button on the confirmation window to overwrite the old calibrator.
7. Reselect the calibrator to be modified, then click the **Edit** button in the bottom right of the window.
8. Select the assay, then select the corresponding Standard field in the Std Concentration column located on the right of the window. Enter the new set point from the Value sheet for the selected chemistry.
9. Click on the yellow **Update** button.
10. Repeat steps 8-9 for all chemistries that use the calibrator.
11. When all entries are completed, click on the **OK** button to register all changes.
12. Click on the **Yes** button.

Manually updating new lots of calibrators whose standard values never change

Note: *Use these steps to update new lots of calibrators whose standard values never change (e.g., ISE Low, ISE High, TDM and drugs of abuse).*

1. Click on the **Calibration** menu button, then the **Install** tab.
2. Click on the calibrator to be modified.
3. Click on the **Add** button located on the bottom left corner of the window.
4. Click on the **Lot No.** field and enter the new lot number.
5. Click on the **Expiration Date** field. Enter the year of expiration in the left field. Enter the month of expiration in the right field, then click **OK**.
6. Click on the **Yes** button on the confirmation window to overwrite the old calibrator.

cobas c 311 analyzer Quick Reference Card

Calibration

Manually editing standard values

Note: *If a Standard Value is reassigned for the lot you are currently using, do not re-download the calibrator lot from* ***cobas*** *link. If the existing lot is downloaded again, the reassigned value does not overwrite the existing value. The reassigned value must be edited manually.*

1. Click on the **Calibration** menu button, then the **Install** tab.
2. Click on the *calibrator* to be modified, then click on the **Edit** button in the bottom right of the window.
3. Select the assay, then select the corresponding Standard field in the Std Concentration column located on the right of the window. Enter the new set point from the value sheet for the selected chemistry.
4. Click on the yellow **Update** button.
5. Repeat steps 3-4 for all chemistry values that are reassigned in the selected calibrator.
6. When all entries are completed, click on the **OK** button to register all changes.
7. Click on the **Yes** button.
8. Update the Lot Calibration for the tests that had edited standard value(s):
   a. Load a new **cobas c** pack for the test.
   b. Perform a calibration within 24 hours of pack registration.

   Note: *A new Lot Calibration must be generated when calibrator standard values are edited.*
9. Recalibrate all **cobas c** packs for the tests that had edited value(s) that are currently on board the analyzer or were registered before the new lot calibration.

Manually requesting calibrations

1. Click on the **Calibration** menu button, then the **Status** tab.
2. Select the desired test from list display.
3. In the Method box on the right of the screen, select the next available calibration method displayed above Span.

   Note: *Span is not an option for Roche assays. If necessary, refer to the package insert to verify the assay's recommended method.*
4. Click on **Save.**
5. All tests that are backlit in green in the Method column now have scheduled calibrations.

   Note: *If there are calibration requests (green) that must be cancelled, refer to the Canceling calibration requests section.*

Canceling calibration requests

1. Click on the **Calibration** menu button, then the **Status** tab.
2. Click on the **Calib. Method** column header to bring both scheduled (green) and recommended (grey) calibrations to the top of the list.
3. Highlight the calibration(s) requiring cancellation.
4. Click on the white button(s) in the **Method** column on the right of the screen.
5. **Do not click on save at this point.**
6. Highlight the methods that are backlit grey and click on the white buttons in the Method column on the right of the screen.
7. Click on **Save** at the lower right of the screen to register all changes. All tests where the Method column is white or backlit in grey are not scheduled for calibration.

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