Laboratory Procedure Manual

**Analyte:** Oral Glucose Tolerance (OGTT)

**Matrix:** Plasma

**Method:** Hexokinase-mediated reaction
Roche/Hitachi Cobas C Chemistry Analyzer

**As performed by:** University of Missouri-Columbia

**Contact:** Dr. Randie Little

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**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:

<table>
<thead>
<tr>
<th>File Name</th>
<th>Variable Name</th>
<th>SAS Label (and SI units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGGT_H</td>
<td>LBXGLT</td>
<td>Two-hour glucose(OGTT) (mg/dL)</td>
</tr>
<tr>
<td></td>
<td>LBDGLTSI</td>
<td>Two-hour glucose (OGTT) (mmol/L)</td>
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</table>
1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas. The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure and liver disease.

Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism or insulin induced hypoglycemia. Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glycosuria, to detect renal tubular defects, and in the management of diabetes mellitus. Glucose measurement in cerebrospinal fluid is used for evaluation of meningitis, neoplastic involvement of meninges and other neurological disorders.

Test principle

UV test
Enzymatic reference method with hexokinase
Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

Reagents - working solutions

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
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

2. SAFETY PRECAUTIONS
Follow all procedures and policies listed in the University of Missouri Hospital & Clinics Safety Manual. Consider all specimens, control materials, and calibrator materials as potentially infectious.

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Wear gloves, lab coat, and safety glasses when handling human blood specimens. Place all plastic tips, sample cups, and gloves that contact blood in a biohazard waste container. Discard all disposable glassware into sharps waste containers. These containers are collected and disposed of twice weekly by University of Missouri waste management personnel.

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

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

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

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

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

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

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

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Plasma: Li-heparin, K2-EDTA and fluoride plasma.

Collect blood by venipuncture from fasting individuals using an evacuated tube system. The stability of glucose in specimens is affected by storage temperature,
bacterial contamination, and glycolysis. Plasma or serum samples without preservative should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand not centrifuged at room temperature, the average decrease in serum glucose is ~7 % in 1 hour (0.28 to 0.56 mmol/L or 5 to 10 mg/dL). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in fluoride tubes.¹

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability (no hemolysis)⁵ 8 hours at 15-25 °C

72 hours at 2-8 °C

Stability in fluoride plasma:⁵ 24 hours at 15-25 °C

Collect urine in a dark bottle. For 24-hour urine collections, glucose may be preserved by adding 5 mL of glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40 % of their glucose after 24-hour storage at room temperature.³ Therefore, keep samples on ice during collection.⁵

CSF
Cerebrospinal fluid may be contaminated with bacteria and often contains other cellular constituents. CSF samples should therefore be analyzed for glucose immediately or stored at 4 °C or -20 °C.³,⁵ Centrifuge samples containing precipitates before performing the assay.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides
Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Equipment and Instrumentation

(1) Instrumentation Cobas C Chemistry System.
(2) General purpose centrifuge.
(3) Refrigerated centrifuge. Temperature range: –8 to 60°C, temperature accuracy: < 2°C, maximum RPM: 8,000 maximum, timer range: 0–99 min in 1-min increments.
(4) Milli-Q Plus ultra-pure water system (Millipore, Bedford, MA).
(5) Pipetman adjustable pipette, 200- to 1000 μL.
(6) Thermolyne Varimix mixer.

B. Reagent handling

Ready for use.

C. Storage and stability

**GLUC3**

Shelf life at 2-8 °C: See expiration date on Cobas c pack label.

On-board in use and refrigerated on the analyzer: 8 weeks

**Diluent NaCl 9 %**

Shelf life at 2-8 °C: See expiration date on Cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

D. Other Materials

(1) Pipette tips, 200- to 1000-μL sizes.
(2) Pipette-Aid.
(3) Pyrex 20-mL disposable pipette.
(4) 5-mL class "A" volumetric pipette.
(5) The following items are all supplied by Roche Diagnostic Systems, Inc. (Indianapolis, IN): sample cups, thermal paper, reagent probe, and 1000-μL reagent syringe, replacement plunger tip for both reagent and sample syringes, sample needles, reagent containers, cuvette segments, sample racks, calibration rack, reagent rack, and thermal printer paper.
(7) Disposable gloves.
(8) Biohazardous waste storage bags and boxes.
(9) Absorbent bench top paper.
(10) Bleach (10% sodium hypochlorite solution)
(11) 1.8 mL Nalgene cryogenic Vials.
(12) Lyophilized serum controls.
(13) NIST SRM lyophilized serum reference material (National Institute of Standards and Technology, Gaithersburg, MD).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Calibration

Calibrators
S1: H₂O
S2: C.f.a.s.
Calibration mode
Linear
Calibration frequency
2-point calibration
• after reagent lot change
• as required following quality control procedures

Traceability: This method has been standardized against ID/MS

Calibration Curve

(1) The Cobas glucose assay uses two calibration point, which is usually analyzed in each run as a sample. The glucose value for the calibrator should be set within manufacturers limits. The instrument requires recalibration if the value of the calibrator is outside the specified limits.

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

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

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

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

B. Verification

(1) In order to verify the calibrator, use glucose (D-glucose) standards. The standards were calibrated against NIST standard reference material SRM, and are stable until expiration date.
(2) Analyzed these standards in a routine assay. Agreement with certified values should be 5%. Frequency of verification is quarterly or whenever it is necessary for troubleshooting the system.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

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

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

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

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

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

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

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

B. Sample preparation

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

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

C. Instrument setup

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

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

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

(4) Streams from both sample and reagent probes should be straight and continuous. The syringes should be free of air bubbles.
(5) Observe the sample needle and reagent probe. They should not appear damaged. Replace them if necessary. Check that the sample tubing is seated properly.

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

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

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

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

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

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

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

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

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

(15) Start the analysis by pressing START.

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

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

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

(19) Turn off the instrument.

E. Recording of Data

(1) Quality Control Data

All replicate values of QC data plus all pertinent assay information (date of analysis, reagent lot number, technician ID, samples ID etc.) are recorded on the Microsoft Access Glucose Daily Diary Log database located on the network drive. The calibrator value is also recorded.

(2) Enter the data under the form “Diary Sheet Entry Form”. The Microsoft Access program will automatically calculate the daily mean and range for each control and determine if a run is accepted or rejected. The current above or below the mean
trend is also calculated. The program will print out a diary sheet for each run and the information is checked and signed by a supervisor.

(3) Analytical Results

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

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

NHANES IV has established a list of comment codes for reporting results. If a result is below the assay detection limit, or a sample is missing, or if the sample volume is less than 200 μL, or the sample is grossly lipemic or grossly hemolyzed, leave the result field blank and record an appropriate comment code in the assay comment field.

(5) A second Data Check List with test results is printed. Test results are verified against the instrument print out. A copy of the data check sheet is kept in the NHANES IV Glucose Data Book at the Diabetes Diagnostic Laboratory at the University of Missouri.

(6) A comma delimited text file (container id.txt) is generated in Hanes4.mdb with an export query. The file follows the format specified by NHANES IV. A copy of the text file is printed and the information is validated against data check sheet.

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

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

F. Replacement and Periodic Maintenance of Key Components

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

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

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

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

G. Application for serum, plasma, urine and CSF

**Cobas c 311 test definition**

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<th>Assay type</th>
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<tbody>
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<td>Reaction time / Assay points</td>
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<tr>
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<tr>
<td>Reaction direction</td>
<td>Increase</td>
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<tr>
<td>Units</td>
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<td>Reagent pipetting</td>
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<td>Normal</td>
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<td></td>
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<td></td>
<td>–</td>
</tr>
<tr>
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<td>15 µL</td>
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<td>135 µL</td>
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</table>

H. Measuring Range

Serum, plasma, and CSF
2-750 mg/dL

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2. This is the maximum dilution for glucose.

Lower detection limit
2 mg/dL

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, within-run precision, n = 21).

I. Quality Control
Serum/plasma/CSF
For quality control, use control materials as listed this laboratory’s Quality Control procedure. The control intervals and limits is adjusted to this laboratory’s requirements. Values obtained should fall within the defined limits. Follow this laboratory’s established corrective measures if values fall outside the limits.

J. Calculation
Roche/Hitachi Cobas C systems automatically calculate the analyte concentration of each sample.

Conversion factors:
- mmol/L x 18.02 = mg/dL
- mmol/L x 0.1802 = g/L
- mg/dL x 0.0555 = mmol/L

9. REPORTABLE RANGE OF RESULTS

A. Expected Values:

Plasma

Fasting

74-109 mg/dL

acc. to Tietz:

Serum, plasma

Adults

74-106 mg/dL

60-90 years

82-115 mg/dL

> 90 years

75-121 mg/dL

Children

60-100 mg/dL

Neonates (1 day)

40-60 mg/dL

Neonates (> 1 day)

50-80 mg/dL

CSF

Children

60-80 mg/dL

Adults

40-70 mg/dL
10. QUALITY CONTROL (QC) PROCEDURES

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

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

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

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

Daily means and ranges of the controls are calculated from 20 interassay determinations. The bias ranges of the daily means are set at ±1 SD or the 67% confidence interval (CI); the warning limits (WL) are the ±2 SD or the 95% CI and the control limits (CL) are the ±3 SD or the 99% CI. For the daily ranges, the bias limit is the mean + 1 SD with warning and control limits set at the mean + 2 SD and the mean + 3 SD, respectively.

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

- The mean for one control from a single run falls outside the 99% confidence limits.
- The means for two controls from a single run fall outside the 95% confidence limits.
- The daily means for one control from eight successive runs (excluding the runs in which the mean is within ±1 SD or the bias range) fall either all above or all below the center line.

The second type of QC chart plots the range of the replicates (the difference between the highest and the lowest value of a single control within a run) and compares it with the established target range, which is the overall mean of daily ranges established by the 20 characteristic runs.
The NHANES guideline declares a system as "out-of-control" if any of the following events occur:

- The daily range for one control exceeds the 99% confidence limit.
- The daily ranges for two controls exceed the 95% confidence limits.
- The daily ranges for one control from eight successive runs (excluding the runs in which the mean is within 1 SD or bias range) are all above the mean line (trend rule).

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

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

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

Criterion: Recovery within ± 10 % of initial value at a glucose concentration of 70.3 mg/dL.

**Serum/plasma**
- Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL).
- Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL).
- 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.
- Drugs: No interference was found at therapeutic concentrations using common drug panels.7, 8

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

**Urine**
- Drugs: No interference was found at therapeutic concentrations using common drug panels.7, 8
For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

NOTE: Glucose values achieved on some proficiency testing materials, when evaluated against a glucose oxidase-oxygen electrode comparison method, demonstrate an approximate 3 % positive bias on average.

**ACTION REQUIRED**

**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/D/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

13. **REFERENCES RANGES (NORMAL VALUES)**

Adults: 74-109 mg/dL

14. **CRITICAL CALL RESULTS (“PANIC VALUES”)**

Critical Value: < 40 mg/dL or > 400 mg/dL

Infants < 1 month: < 40 mg/dL or > 300 mg/dL

**Critical results must be repeated and verified.**

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 to 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

Please see the following page.
### 2013-2014 Summary Statistics and QC Chart for Two hour oral glucose tolerance (OGTT)

<table>
<thead>
<tr>
<th>Lot</th>
<th>N</th>
<th>Start Date</th>
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OGTT Glucose in Plasma
NHANES 2013-2014
References: