Laboratory Procedure Manual

Analyte: **Triglycerides**

Matrix: **Serum**

Method: **Cobas 6000 Chemistry Analyzer**

As performed by: Advanced Research and Diagnostic Laboratory
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Important Information for Users

The University of Minnesota/ARDL 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.

Data File Name	Variable Name	SAS Label
TRIGLY_H	LBXTR	Triglycerides(mg/dL)
	LBDTRSI	Triglycerides(mmol/L)

1. **SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE**

   A. Clinical Relevance

   Triglycerides are fatty acid esters of glycerol that have three hydroxyl groups. Because they are insoluble in water, the triglycerides are transported with other more polar lipids. Elevated triglyceride measurements are associated with diabetes mellitus, pancreatitis, alcoholism, glycogen storage disease, hypothyroidism, nephrosis, pregnancy, use of oral contraceptives and gout. Triglyceride levels are decreased in hyperthyroidism, use of certain lipid-lowering drugs and malabsorption syndrome.

   B. Principle

   The Roche/Hitachi Cobas 6000 analyzer series is a fully automated, random-access, software- controlled system for immunoassay and photometric analyses intended for qualitative and quantitative *in vitro* determinations of a wide variety of tests. The Cobas 6000 analyzer is optimized for workloads using a combination of photometric and ion-selective electrode (ISE) determinations (c501 module), and electrochemiluminescence (ECL) technology in the immunoassay analysis module (e601 module).

   The ISE system is used in the quantitation of sodium, potassium and chloride. The photometric system can measure colorimetric or immunoturbidimetric reactions utilizing end point or kinetic (rate) absorbance measurements. Test ordering and execution on the Cobas 6000 and data entry in the StarLIMS host computer system may be done manually or these tasks may be executed via a barcode-based bi-directional interface. The Cobas 6000 can utilize both of these two systems simultaneously.

2. **SAFETY PRECAUTIONS:**

   A. Daily Safety Precautions.

   All personnel working in the laboratory must wear gloves and laboratory coats. Laboratory coats are to be kept snapped. Lab coats must meet OSHA compliance CPL2-2.44D. Splash and spray resistant fabric that is also antistatic is required. Gloves are removed when leaving the immediate work area or when entering offices within the immediate work area. All used gloves, vials, pipettes and other items that come in contact with specimens are disposed of in a Biohazard box lined with a red plastic bag.   Work benches are cleaned at the end of each day, or during the day as needed, with germicidal disposable wipes.

   B. Blood Handling.

   The improper handling of blood samples from patients with infectious diseases, e.g., hepatitis or HIV, can lead to infection of staff that draw, handle, analyze or store such samples. Transmission can occur by ingestion, inhalation or direct contact, and staff must exercise care when handling blood samples. <u>Always wear liquid impermeable gloves (e.g., nitrile or plastic) when handling biological samples.</u> The use of latex gloves is not allowed due to concerns for personnel having or developing latex sensitivities. <u>Never pipet samples by mouth</u>. Avoid contact with serum. Cover any scratches or cuts on fingers and hands and wear gloves before handling serum. Store all samples in sealed containers. In order to minimize the formation of aerosols, and to prevent evaporation, do not leave

samples open to the atmosphere longer than necessary.

It is about <u>30 times easier</u> to become infected with hepatitis than with HIV through sample mishandling, and it has been recommended that the usual precautions for handling blood specimens to prevent hepatitis infection serve as a guide to prevent AIDS infection as well. Handle all specimens as if you know them to be infectious. All staff should adhere to the CDC Guidelines for Prevention of HIV Infection in Health Care Workers.

C. Spills.

The contaminated area is cleaned with a solution of sodium hypochlorite (bleach: water, 10:100, v/v) and the wipes are disposed of in a red biohazard container.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

NHANES results are entered unto a spreadsheet provided electronically by WESTAT, Inc for NHANES.

Within the University of Minnesota/ARDL computer network, access the spreadsheet: click on Computer → S: drive → ARDL Share → NHANES Lipids.

Choose the file named with the corresponding box number.

Results are downloaded from StarLIMS. Some parameters are manually entered: receipt date, run number, specimen comments. See separate procedure "NHANES Data Management (Revised)" from University of Minnesota/ARDL for additional detail.

The spreadsheet will be sent electronically by the ARDL LIS contact person.

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

A. Specimen handling

Use serum or plasma (EDTA and heparin anticoagulants are acceptable) for the procedure. Other anticoagulants are unacceptable. Serum or plasma is stable for 5-7 days at 4ºC, and longer at –70ºC.

Serum or plasma specimens that have been frozen are sometimes prone to excessive precipitate formation. These specimens should not be centrifuged as that could cause layering of the lipids in the specimen. Since lipids tend to rise during centrifugation, this could lead to falsely elevated results. This is because the sample probe on the COBAS aspirates as soon as it contacts the liquid surface. Excessive particulate matter may be removed by inserting a wooden stick into the specimen to pick up the particles, or by drawing the serum or plasma through a coarse pipet-tip filter.

Bilirubin does not interfere up to an I index of 60. Hemolysis does not interfere up to an H index of 400. There is a poor correlation between the triglyceride concentration and visible lipemia. Specimens with an exceptionally high triglyceride concentration (>3000 mg/dL) may produce a normal result. Therefore, very lipemic specimens should be manually pre-diluted 1:5 or assayed on decreased sample volume.

Minimum sample volume: 100 uL (includes dead volume)

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

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

A. Instrumentation

1. Roche/Hitachi Cobas 6000 Analyzer. Roche Diagnostics, 9115 Hague Road, P.O. Box 50457, Indianapolis, IN, USA 46250-0446.

2 . On the c501 module the Cobas 6000 utilizes spectrophotometric and ion-specific electrode measuring systems. The instrument utilizes reusable optically pure plastic reaction cells that are changed on a monthly basis. The reaction cells are automatically washed by the instrument after completion of the test cycle. Sample and reagents are added to the reaction cells at specific timed intervals, varying by the program parameters defined for each test. Most methods utilize two reagents, but a few use one. All reagent bottles have a uniquely barcoded ID label on them so they are recognized when loaded onto the instrument.

3. The Cobas 6000 measures the reagent volume in the bottle as it is withdrawn, so the instrument provides a real time update on the number of tests available in each of the bottles.

4. The measuring system in the e601 module is based upon the principle of electrochemiluminescence (ECL). Single-use cuvettes and sample tips are used instead of re- usable ones. Similar to the c501 module, reagent vessels are uniquely barcoded and the test count is updated in real time. In general, each reagent vessel consists of three reagents— streptavidin-coated microparticles, biotinylated antigen or sandwich antibody, and ruthenium- labelled antibody.

5. Tests can be ordered manually or they can be executed by use of the bi-directional interface connected to the starLIMS host computer. Similarly, reporting can be achieved through the interface, by manually keying the results into StarLIMS from the instrument's hard copy printout, by data entry into a spreadsheet or a website, or by manipulation of the instrument's downloaded data file. Which avenue is chosen for these functions is dictated by the parameters of specific studies.

6. Purified water supply. The Cobas 6000 requires a continuous supply of purified water. The system used by the Cobas 6000 is the Millipore Elix Gulfstream Clinical System 35c. Millipore SAS 67120 Molsheim, France. The water is filtered from the reservoir through the inlet solenoid valve to the Progard TL Pretreatment Pack. It then passes through the Reverse Osmosis Permeate Divert Solenoid Valve, a strainer, and a 254 nm UV Lamp. The water also passes through the Q-Gard TL Polisher Pack and PrePak L1 Pretreatment Pak.

7. The water system should be checked daily to indicate the distribution is ≥10 mg. Every three months the RO membrane must be cleaned, and every four months the Progard TL Pretreatment Pack, Q-Gard TL Polisher Pack, and PrePak L1 Pretreatment Pak require replacement. The Automatic Sanitization Module requires replacement after two years. Service can be requested by calling (888) 645-5478, the system serial number is: F3CA36895B.

B. Other Materials

1. Sample cups (micro). Roche product #05085713.
2. Sample cups (standard). Roche product #10394246.
3. Reaction cell cuvette segments. Roche product #04854241 (24 segments/box). Eight segments complete the entire rotor). Perform cell wash and cell blank functions after installation. Change cuvettes monthly.
4. Printer paper, 8.5 x 11 inch. Various sources including Bose Multipurpose Paper.
5. Printer cartridge. HP Laserjet CE505A, black. Hewlett-Packard Company
6. COBAS c pack MULTI. Roche product #04593138190.

C. Reagent Preparation

1. Cell Wash Solution II/Acid Wash. Roche product #04880307190 (1.8L bottle). No preparation required. Solution of formic acid, citric acid and nikkol BT-9. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 12 weeks after opening. This solution is automatically drawn by the Cobas 6000 while cleaning reaction cuvettes during analysis.

2. Cell Wash Solution I/NaOH-D. Roche product #04880285190 (1.8L bottle). No preparation required. Solution of sodium hydroxide (1N). Store at room temperature. Stable until expiration date on bottle, the on-board bottle stability is 10 weeks after opening. This solution is automatically drawn by the Cobas 6000 while cleaning reaction cuvettes during analysis.

3. ECOTergent/Hitergent/Eco-D. Roche product # 06544410190 (12 bottles/box). No preparation required. Solution of ethanolamine, hexahydro-1,3,5-tris (Betahydroxyethyl) triazine and nonidet P-40. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 12 days after opening. Hitergent is an on-board reagent automatically drawn by the Cobas 6000 during the daily incubator bath exchange.

4. ProCellM. Roche Product # 04880340190 (2 L bottle). No preparation required. Solution of Tripropylamine (TPA) and Oxaban A. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 5 days. This is a buffer solution that is used for conditioning the electrodes, transporting the assay reaction mixture,

washing the streptavidin-coated microbeads and signal generation. ProcellM is automatically drawn by the Cobas 6000 during analysis.

5. CleanCellM. Roche Product #04880293190 (2L bottle). No preparation required. Solution of Potassium Hydroxide and Polidocanol. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 5 days. The solution is automatically drawn by the Cobas 6000 to clean the measuring channel after each measurement and conditioning the electrodes.

6. PreCleanM. Roche Product #03004899190 (600 mL bottle). No preparation required. Solution of Polidocanol and OxabanA. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 4 weeks. This is a phosphate buffer that is used to wash and resuspend microbeads during the pre-wash step. PreCleanM is automatically drawn by the Cobas 6000 during analysis.

7. ProbeWashM. Roche Product #03005712190 (70 mL bottle). No preparation required. Solution of Polidocanol and Potassium Hydroxide. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 4 weeks. The solution is used to clean the reagent probe during special wash steps and at the end of the run.

8. ISE Cleaning Solution/Elecys SysClean. Roche Product #11298500160 (100 mL bottle). This is a sodium hydroxide and sodium hypochlorite solution. Store at 2-8C. The solution is stable up to the stated expiration date when stored at 2-8 °C.

D. Preparation of Quality Control Materials

1. Reagents. Triglycerides/Glycerol Blanked reagent system. Roche Product #11877771216, 12 x 120 tests. Using the provided adapter, connect one Bottle 1a (enzymes) to one Bottle 1 (buffer). Mix by gentle inversion. Using a different adapter, connect one Bottle 2a (lipase/4-aminophenazone) to one Bottle 2 (buffer). Mix by gentle inversion. Allow 15 minutes for complete dissolution. Transfer 15 mL of Reagent 1 to position B of a cobas c pack MULTI. Transfer 15 mL of Reagent 2 to position C of the same cobas c pack MULTI. Leave position A empty. Label the pack using the label provided in the reagent kit. Load the pack onto the COBAS in the routine manner.

2. Calibrators. Calibrator for Automated Systems (C.F.A.S.). Roche Product #10759350360. Volumetrically dilute one bottle of calibrator with 3.0 mL deionized water. Mix gently and allow to dissolve for 30 minutes at room temperature. Precimat Glycerol. Roche Product #10166588130. No preparation required. Use as the zero calibrator for this assay.

3. Quality control materials. Two levels of controls are assayed daily. Check current QC records for lot in use and acceptable values.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

There is a variety of calibration models used on the Cobas 6000. There are factored methods, blank calibrations, two-point calibrations and multi-point calibrations. The type of calibration is dictated by the Roche application parameters for each method. This method utilizes a one-point model. The calibrator material may be a Roche product, an in-house preparation, or a product from another company. A new set point value is

typically assigned whenever a calibrator lot number changes. If the calibrator is a Roche product, the updated set point value must be downloaded via the COBAS Link. This is a direct, web-based link from the Cobas 6000 to the Roche database of lot-specific calibrator and control values. If the set point change is for a non-Roche product, then the update must be performed manually.

Frequency of calibration is dictated by an automatic, time-dependent re-calibration built into the application parameters for each test, and by observing the quality control data. All methods do not have an automatic time-out calibration feature. Details for each assay may be found in specific procedures and in the application parameters on the Cobas 6000. Acceptable accuracy and precision limits are defined in each chemistry parameter file. This assay requires recalibration for every new reagent pack that is placed on the COBAS.

Verification:

Calibration verification is an ongoing process conducted through the measurement of CDC survey specimens (values assigned by reference methodology), and an accuracy-based lipid survey from CAP (College of American Pathologists). Linearity is also assessed semi-annually.

8. **PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS**

A. Theory of Operation

Samples for ISE determination and photometric measurement can be directed by the user or an interfaced order. Each ISE specimen is prediluted by the instrument in one of 160 reaction cells. The diluted specimen is then measured in the ISE measuring system. Samples for photometric analysis are measured by the photometer. The photometer measures either endpoint or rate reactions that have occurred in the reaction cell with absorbance changes measured using discrete wavelength settings. Following completion of ISE and photometric reactions, the cell rinse unit washes the reaction cell, and the cell is re-used. All analyses occur at 37⁰C.

Samples for immunoassay determination are directed by the user or an interfaced order to the e601 module for sampling. A new disposable tip is used for every pipetting sequence, preventing carryover. Samples are pipetted into one of 54 single-use sample cups in an incubator disk that is maintained at 37⁰C. Two individually calibrated ECL detection cells are the central components performing measurement of samples. Each detection cell contains a photomultiplier tube and flow-through measuring channel. Two test principles are used: competitive principle (very small analytes) and sandwich principle (larger analytes). Following analysis, sample cups and tips are disposed of in a solid waste container on the instrument.

B. Specimens

Follow all usual precautions for obtaining a specimen by venipuncture. To determine the correct specimen type, refer to the specific assay procedure. Follow

ARDL's Laboratory Safety Manual isolation and standard precautions for blood and body substances.

The approximate dead volume for the Hitachi standard cup is 100 uL. For the Hitachi micro cup the dead volume is 50 uL.

Specimens may also be direct-sampled in the original storage vial. These vials are typically prepared by field center laboratories after collection of specimens at their site. Because they are frequently very small with small volumes of specimen, care must be taken that these vials are very thoroughly mixed prior to sampling. Since mixing or vortexing can cause bubble formation (which interferes with the Cobas 6000 sample detection system), care must be taken to remove these bubbles before analysis begins. This can be done by poking the bubbles with a wooden stick, or by a short (1 minute) centrifugation at 1,500 x g. However, if lipids are being measured centrifugation is not an option because the triglycerides could layer out into the surface of the specimen, resulting in an artifactual increase in their concentration. Storage vials come in a variety of shapes and sizes, and the clip design of the Cobas 6000 sample racks accommodates most of them. All specimens for immunoassay testing must come to room temperature prior to analysis.

C. Instrument Overview

A general description of the instrument and its components is found in Section A of the Cobas 6000 analyzer series Operator's Manual, Version 5.

D. Instrument Setup

The instrument should be placed in Sleep mode when not in use for long periods (e.g. overnight). This prolongs the lamp life and other essential components. When in sleep mode the reagent compartment remains refrigerated, but most other functions are turned off. The Cobas 6000 is programmed to re-boot every weekday morning at 0600, and Monday at 0500. It does not automatically reboot on weekends. If the Cobas 6000 needs to be turned on prior to its pre-programmed start time, follow the screen prompts to activate the system. In this case a Daily Pipe (a series of mechanized boot-up processes) must be manually ordered.

The Cobas 6000 has scheduled daily, weekly, bi-monthly, monthly, quarterly and as-needed maintenance. These tasks are listed in the check-off chart at the instrument, and described in detail in Section C of the Operator's Manual, Version5. Perform all scheduled maintenance before beginning testing.

E. Loading Calibrators and Controls

For the c501 module, each calibrator and control has a defined location on either the black calibrator sample racks or white control racks. A complete posting of all of these assigned locations is available on the Cobas 6000 computer. The assigned locations are also marked on the calibrator and quality control racks. Either a standard sample cup or micro sample cup is acceptable to hold the calibrators and quality control material. Fill the sample cup with enough volume to complete the full calibration sequence (this will vary by method; some multi-point calibrations will sample the standard several times in order to make serial dilutions, while in a two-

point calibration, the standard is only sampled in duplicate) or control testing. Most calibrations require water as the "zero" standard, but the triglycerides assay uses glycerol.. Make sure it is loaded.

The sample cups are loaded into the calibrator and control sample racks. If using 2 mL Sarstedt vials for a control, the Cobas 6000 will not be able to detect the vial if it is seated too low in the rack. The bottom of the 2 mL vial should be seated to approximately the bottom of the metal prongs in the rack. Take care to make sure the vial is seated far enough down so it is not jarred from the rack during transport on the Cobas 6000.

For the e601 module, CalibratorSet and ControlSet vials from Roche are used, and each vial has a barcode provided in the calibrator or control kit. However, due to inconsistent barcode detection by the instrument scanner, permanent locations have been assigned for all of the e601 calibrators and controls. Because of this permanent location assignment, all barcodes must be marred by drawing a horizontal line through the barcode with a Sharpie marker. If this is not done, the instrument may detect the barcode, and it will reject the rack.

All calibrator and quality control material must be at room temperature prior to placement on the instrument. The calibrator and control vials are placed in black calibrator and white control racks with florescent pink stickers. Take care that the calibrator and quality control vials are seated firmly in the rack and the cap is opened to a perpendicular position prior to loading on the instrument. Take care that the vials are correctly oriented in the rack so the cap opens to the left (when viewing the front of the rack).

See specific protocols for the assigned calibrators and controls to be used for each test.

F. Requesting a Calibration

1. At the home screen, click or touch screen on <Calibration> tab.
2. Click or touch screen on <Status>. A list of all the Cobas 6000 tests appears. If more than one bottle set of reagents is on-board, a separate listing will appear for each set.
3. Click or touch screen on the tests to be calibrated.
4. In the "Method" box on the right side of the screen, select (click) the appropriate type of calibration to be performed on the selected test. The correct type of calibration for each method can be found in the specific test protocols located in the Cobas 6000 Applications folder. Most c501 methods utilize a two-point calibration, while all e601 methods utilize a full calibration. Generally, if a screen button is white, that means it is active/available. Yellow indicates completion, gray indicates inactive/unavailable. Make sure that the Method box is white before clicking on Save below.
5. If a calibration has timed out, or if there has been a reagent lot change, this information appears in the Cause column. In these cases just highlight the test, skip the Method box, and touch or click on <Save>.
6. Click or touch screen on <Save>.
7. Failed calibrations will generate an error message by the Cobas 6000. For the c501 module, the two most common flags found in a failed calibration are SENS and DUP.

a. SENS (sensitivity error) occurs when the difference in absorbance between the zero standard and measuring standard does not fall within a method-specific, defined range. Typically, the absorbance difference is too small, and this usually indicates a deteriorated reagent. Replace the reagent cassette, and repeat the calibration.

b. All calibrators are assayed in duplicate. DUP (duplication error) occurs when the pair of measurements at the zero and measuring points do not agree satisfactorily with each other. In this case simply repeat the calibration. If the error occurs again, consider sources of imprecision (sample probe, syringe leakage, bubbles in reagent, etc.).

For the e601 module, the most common flags fond in a failed calibrator are Monotony of Curve, Deviation of Duplicates and Factor.

a. Monotony of Curve error occurs when calibrator values do not fall in ascending (sandwich) or descending (competition principle) order. To troubleshoot, if a calibrator was reconstituted or aliquoted, make sure it is placed in the correct vial with the appropriate label. Check ProCell expiration date, recalibrate.

b. Deviation of Duplicates error occurs when the difference between duplicate signal measurement (signal 1 & 2) is too large. You will see "NG" under the Dupl. on the print out. To trouble shoot, check for bubbles in the reagent or calibrators and make sure reagent and calibrators were at room temp. Check the ProCell expiration also, and repeat the calibration.

c. Factor error occurs when comparing a "R" reagent calibration to the original "L" lot calibration and the calibrator factor does not fall between 0.8 – 1.2. To troubleshoot, make sure that the reagent and calibrator are at room temperature and free of foam and bubbles. It is possible that the stored calibration was a bad calibration. First try repeating the calibration, then place a new pack and update the lot calibration.

d. Other calibration topics are found in the Operator's Manual, Section B, Chapter 12.

G. Loading Reagents (Operator's Manual c501 Section A-60, e601 Section A-86)

Review available reagents by <Reagents>, <Status>. Sort the reagents by clicking on Available Tests. This lists the reagents in ascending order of the number of tests performable with the reagents currently on the Cobas 6000. You can sort by the c501 or e601 module by selecting it from the Module: dropdown just above test name. When a test is highlighted on this list, the reagent bottle locations, number tests remaining and stability by bottle, are shown in the window on the right side of the screen.

c501

The principle reagent containers for the c501 modules are reagent packs that contain up to three reagent vials. Reagent packs are stored in a refrigerated regent compartment on the c501 that stores up to 60 reagent cassettes. Most test methods use two reagents, though some use only one. Generally, R1 is a buffered reagent that establishes the optimum pH and reaction conditions for the test, and R2 has the enzymes and/or chromogenic components that complete the reaction. If a test volume greater than the capacity of one reagent container is anticipated, additional bottles may be placed on the instrument. If an automatic calibration is not required on bottle change, or if the new bottle set was calibrated when it was placed on the instrument, then the Cobas 6000 will automatically begin pipetting a new bottle once the previous bottle is empty.

Roche-provided reagent packs have a two-dimensional, barcoded ID on one side. This

barcode contains lot number, test code, expiration date and available test count information. When loading reagent packs into the cassette loading area on the c501, the barcode must face to the right. As a reminder, there is a diagram illustrating this on the loading stage. For some Roche tests (ex. B2M and Triglyceride-Glycerol Blank), the reagent does not come configured as a reagent cassette. The operator must transfer the reagents from the bottle sets to the appropriate positions (A, B, C) in an empty cassette/pack called a Cobas c pack MULTI. Refer to the analyte package insert for detailed instructions. Barcodes for these reagents are available in the reagent box. After loading the reagents into the MULTI cassette, affix an assay specific barcode to the MULTI pack covering the original barcode. Only *one* barcode should be visible on the cassette. The cassette is now ready for loading.

Open channel tests also utilize the Cobas c pack MULTI. These tests have specific reagent volumes that are loaded into specific wells in the MULTI cassette. While in Standby mode, to load an Open Channel reagent pack, go to Reagent, Setting. Make sure the c501 module is selected and click on "Open Channel" in the bottom right corner. A window will appear, and then select the test being loaded and click Reserve, then OK. Load the MULTI pack (barcode facing right) in the cassette loading area.

Unloading and Reloading Cobas c packs: The system counts down each cassette's initial number of available tests each time it pipets out of the cassette. If a pack is unloaded and later reloaded, the system recognizes the cassette and begins counting down at the point when it was unloaded, assuming the cassette's reagent volume remains unchanged. However, if a reagent cassette is "dumped", that cassette cannot be returned to the instrument.

e601

The principle reagent containers for the Cobas e601 module is the Cobas e pack. This pack consists of three separate capped reagent containers. Each pack is equipped with a barcode label that contains reagent, lot number, control, calibration, expiration and stability information. The Cobas e packs are loaded into one of the 25 places on the e601 reagent disk. To prevent reagents from evaporating, the e601 has a reagent cap open/close step that is performed during reagent pipetting. To load reagents, make sure the e601 module is in the standby mode and the microbead mixer is not over the reagent compartment. Open the blue top by turning the key, turn the e601 reagent compartment handle to the "open" position and lift up, removing the lid. On the Cobas e pack being loaded, open each of the three caps to the partial open position and place the pack in the carousel. Each pack is keyed, so it only fits in one position--with the white cap in the outermost position. After loading the reagents replace the carousel lid and move the handle to the "lock" position. The reagent barcode reader will register all of the reagents on the reagent disk.
In the event automatic registration of an e601 reagent fails, the reagent pack barcode can be manually registered. To do this, go to Reagent, Setting, Manual Registration to open the Manual Registration window. Type the barcode of the Cobas e pack and chose OK.

H. Loading Specimens: (Operator's Manual Section B-52)

Given that ARDL receives specimens from so many different sources, in many different containers, and provides results via different mechanisms (report from host, manual entry to spreadsheet, manual entry to website, processing of the instrument data download), there

are multiple ways to load specimens and order testing on the analyzer. Since the NHANES
study utilizes barcoded ID numbers for bidirectional interfacing, that process will be
described in Section I below.

Prior to placing specimens on the instrument, it is mandatory that all specimens be
thoroughly mixed. Most specimens analyzed in ARDL have been frozen, so this step is
critical. Mixing sometimes causes surface bubbles to form, and these must be remedied
before sampling.
Poking the bubbles with a wooden applicator stick is recommended. Urine specimens must
be centrifuged following mixing.

All specimens analyzed on the e601 module must be at room temperature before they are
placed on the instrument.

I. Operation of Assay Procedure

To preserve the integrity of the label on the original sample vial, the barcoded StarLIMS label
is instead affixed to a 16x75 mm support tube. An insert dropped into this tube allows the
specimen vial to rest inside the support tube at a level that allows accurate sampling. Care
must be taken to match the NHANES ID on the vial to the NHANES ID on the StarLIMS label.

It is not necessary to "connect" the Cobas 6000 to the host. This connection is always in
place, with a Data Innovations middleware system serving as a buffer between the Cobas
6000 and STARLIMS.
The desired tests are ordered in the host computer system, STARLIMS.
A container ID (CID) is generated by the system, and a label is produced with the barcoded
CID on it. This label is placed on the support tube.
After arrival of the specimen at the analyzer, and following mixing and/or centrifugation, the
labeled support tube/vial combination is placed into a proper rack.
When loading the vials onto the sample racks, the caps are removed and stored in a
sequential system to allow re-capping of the vials with the same cap following analysis. A
board with numbered holding positions in the processing area facilitates this process.
The barcode on the support tube must face out through the groove in the rack.
If there is adequate reagent onboard and the necessary calibrations and controls have been
successfully completed, load the sample racks onto the instrument. If the access light is
green on the left side of the loading area, lift the lid of the sample loading compartment,
remove the tray, and place the racks onto the tray. Since the tray (and the slot in the racks) is
offset, there is only one way to load them onto the instrument.
After the racks are loaded, return the tray to the loading station, close the lid and click
<Start>.
The Start Conditions window appears. Click the big <Start> to begin the run.
The sample rack arm will move from a vertical position to horizontal, and sweep the sample
racks into the barcode scanning station. From there, the racks enter the holding carousel
prior to sampling on the c501 or e601.
After this process has begun, additional specimens may be loaded. Tests may be added
anytime, but the Cobas 6000 will not allow the Start button to be activated if it is still in
Operation mode.

J. Instrument Shutdown

After bringing the instrument to Standby mode, and successfully transferring the data to
the mass storage and S: drive locations (see separate procedure), the Cobas 6000 is

ready for activation of the Sleep Pipe (automated, mechanized sequence of shutdown processes). First, load the designated green rack as follows, using standard sample cups, half-filled:

Pos 1: MultiClean

Pos 2: Sys Clean

Pos 3: Leftover serum or serum-based control material

Place the rack on the sample loading tray.

Then request the Sleep Pipe:
1. <Utility>
2. <Maintenance>
3. <Pipe Functions>
4. <Sleep Pipe>
5. <Execute>

The instrument samples the green rack elements, and completes the Sleep Pipe functions in approximately 45 minutes. It then enters sleep mode until re-starting at the pre-programmed time the following morning.

K. Recording Data

Control and calibration results will automatically print out on the remote printer connected to the Cobas 6000. Patient result printouts must be requested on the Cobas 6000: <Workplace>, <Data Review>, highlight desired records, <Print>, <Print>.

Hard copies of patient data should be generated only if reviewing the data for verification in STARLIMS, or if the results are to be manually entered into a spreadsheet or website. If the data is to be reported using an instrument download, then it is not necessary to print that data.

Detailed STARLIMS instructions may be found in specific STARLIMS protocols, but the general process for automated entry is thus:

1. Log in with personal user ID and password.
2. <Start Batch>
3. Select appropriate batch category from drop down menu.
4. <Close Batch>
5. <LifeCycle icon>
6. <Result/Finish Batch>
7. Select appropriate batch category/number from drop down menu.
8. Review data. Accept, correct or comment as necessary.
9. <Finish Batch>

Manual data entry in STARLIMS is done via the <Order/Result Review> option on the Dashboard. Select <Advanced>, then enter the CID of interest. The entry fields appear in the lower portion of the screen. After data entry, select <Finish Result>, then <Release Pending>.

Data entry into spreadsheets is typically accompanied by an additional tab for a Data Dictionary where details regarding the methodology can be provided. This information is

available in the ARDL Data Dictionary folder on the S: drive.

9.   REPORTABLE RANGE OF RESULTS

The technical range for the triglycerides/glycerol blank assay is 9 – 885 mg/dL. Values exceeding 885 mg/dL are automatically diluted 1:5.5 by the analyzer, resulting in a reportable range of 4868 mg/dL.

10. QUALITY CONTROL (QC) PROCEDURES

Two levels of controls are assayed daily, in duplicate. Control status is assessed before testing begins, and then again following completion. One level is a pool of normal human serum, stored in individual vials at -80°C. A new vial is removed from freezer daily for use. The other control is a lyophilized commercial product stored at 2-8°C. Acceptable ranges are established prior to placing a control into use, with the ranges based upon historical precision parameters established in ARDL.

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

Do not release patient data when control errors occur.

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

According to Roche, no interference was found at therapeutic concentrations using common drug panels. Exception: Intralipid is directly measured as analyte in this assay and leads to high triglycerides results. Excessively lipemic samples should be manually pre-diluted before analysis. Bilirubin does not interfere up to an I index level of 60. Hemolysis does not interfere up to a H index level of 400.

13. REFERENCE RANGES (NORMAL VALUES)

The adult reference range is 0-150 mg/dL, based upon NCEP recommendations.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Early Reporting Results for NHANES:
Notify the NHANES Medical Officer of any critical result. The contact person will report these results as soon as possible.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Samples are received frozen and stored at -80°C until testing is performed

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

Samples are held at -80ºC in the freezer.

17. **TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)**

For routine reporting, see section 3.

Early Reporting Results for NHANES:

Notify the NHANES contact person. The contact person will report these results as soon as possible.

18. **TRANSFER OR 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 graphs**

See following pages.

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
15301_LBCTRGH1	29	21JAN13	12AUG13	186.6207	3.7265	2.0
P_LBCTRGL1	9	21JAN13	18MAR13	125.8889	2.3688	1.9
Q_LBCTRGL1	96	25MAR13	02FEB15	98.5000	3.7781	3.8
27611_LBCTRGH1	76	19AUG13	02FEB15	178.8947	3.9212	2.2
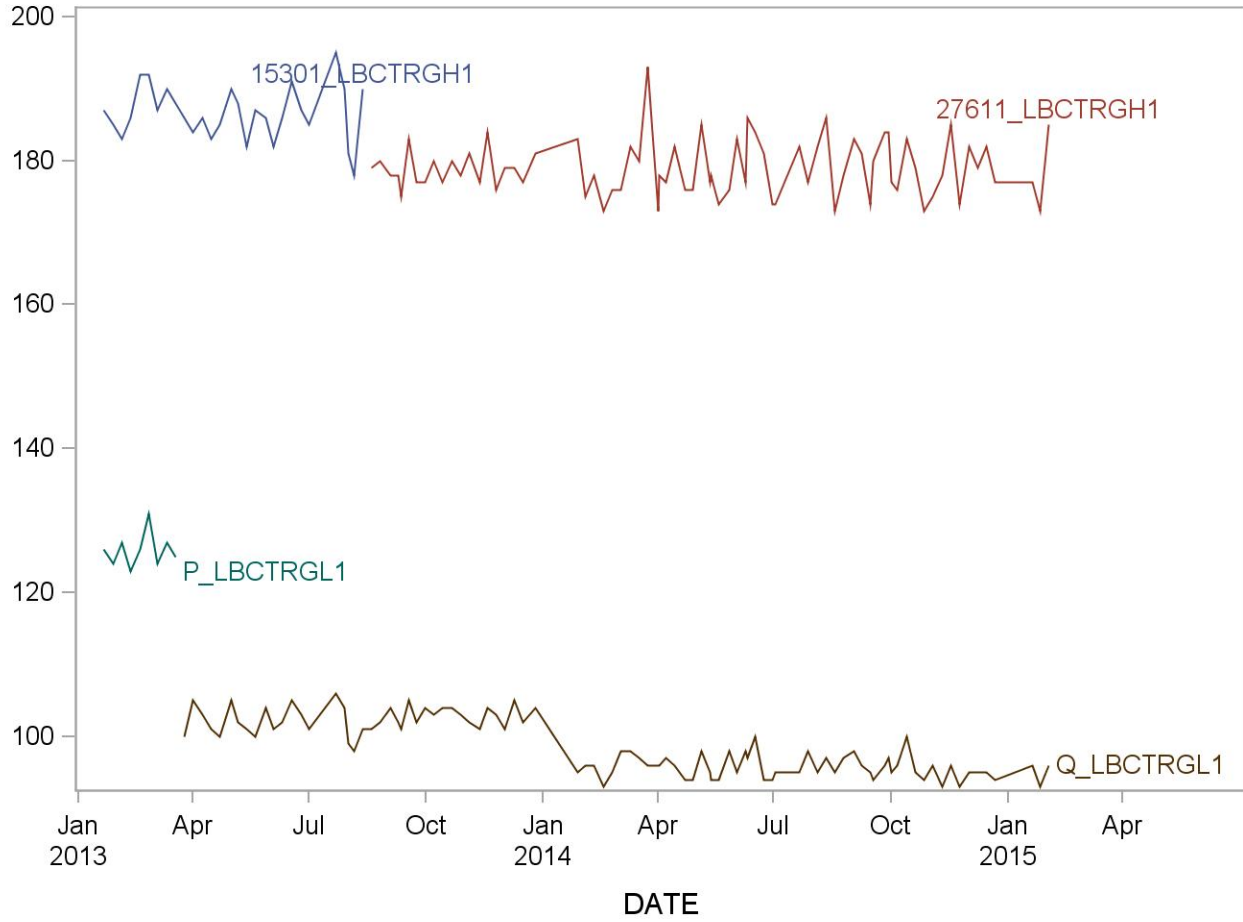
20. REFERENCES

Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.

Roche Trig/GB reagent package insert. Version 2014-06, V8.0. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.

Roche Calibrator for Automated Systems (C.F.A.S.) package insert. Version 2011-08, V6. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457