

Laboratory Procedure Manual

Analyte: **HDL- Cholesterol**

Matrix: **Serum**

Method: **Cobas 6000 Chemistry Analyzer**

Method No.:

Revised:

As performed by: **University of Minnesota
Advanced Research and Diagnostic Laboratory (ARDL)
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Important Information for Users

The Advanced Research and Diagnostic Laboratory (ARDL) periodically refine 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:

Data File Name	Variable Name	SAS Label
HDL_J	LBXHDD	HDL-Cholesterol (mg/dL)
	LBDHDDSI	HDL-Cholesterol (mmol)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

In this method a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. These complexes are not reactive with the measuring reagents added in the second step. With addition of reagent 2, HDL-cholesterol esters are converted to HDL-cholesterol by PEG-cholesterol esterase. The HDL-cholesterol is acted upon by PEG-cholesterol oxidase, and the hydrogen peroxide produced from this reaction combines with 4-amino-antipyrine and HSDA under the action of peroxidase to form a purple/blue pigment that is measured photometrically at 600 nm (secondary wavelength = 700 nm). When the cholesterol measuring enzymes are modified with PEG, they are preferentially more reactive with HDL-cholesterol than the other cholesterol fractions. This is an endpoint reaction that is specific for HDL-cholesterol. This 3rd generation method differs from 2nd generation assays in the type of buffer used in the reagents, and the concentration of the reagent components. The basic reaction principle is unchanged.

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 with a solution of sodium hypochlorite (bleach: water, 10:100, v/v) and then covered with plastic-backed white paper.

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. Always wear liquid impermeable gloves (e.g., nitrile or plastic) when handling biological samples. The use of latex gloves is not allowed due to concerns for personnel having or developing latex sensitivities. Never pipet samples by mouth. 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 aerosols, do not leave samples open to the atmosphere longer than necessary.

It is about 30 times easier 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 box.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

NHANES results are entered into 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

Use serum or plasma (EDTA and heparin are acceptable) for the procedure. EDTA specimens yield slightly lower results (3-6 per cent) than serum due to migration of water from cells to the plasma. Other anticoagulants are unacceptable. Serum or plasma is stable for seven days at 4°C, and longer at -70°C.

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 6000 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 index of 30. Hemolysis does not interfere up to an H index of 1200. Lipemia does not interfere up to an L index 1000.

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

B. 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. The water system should be checked daily to indicate the distribution is ≥ 10 mg. Every three months the RL 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.

C. Cobas 6000 system reagents.

- 1) Cell Wash Solution II/Acid Wash. Roche product #4880307 (2L bottle). No preparation required. Solution of formic acid, citric acid and nikkol BT-9. Store at room temperature. Stable until expiration date on bottle, 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 #4880285 (1800 mL 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) 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) ECOTergent/Hitergent/Eco-D. Roche product # 6544410 (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.
- 5) ProCellM. Roche Product # 04880340 (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.
- 6) CleanCellM. Roche Product #04880293 (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.
- 7) PreCleanM. Roche Product #03004899 (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.
- 8) ProbeWashM. Roche Product #3005712 (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.

- 9) ISE Cleaning Solution/Elecys SysClean. Roche Product #11298500 (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.
- 10) Sample cups (micro). Roche product #05085713.
- 11) Sample cups (standard). Roche product #10394246.
- 12) Printer paper, 8.5 x 11 inch. Various sources including Bose Multipurpose Paper.
- 13) Printer cartridge. HP Laserjet CE505A, black. Hewlett-Packard Company.
- 14) Reagents and calibrators. See specific assay procedures.
- 15) Quality control materials. Two levels of controls are assayed each day that a specific test is performed. 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

The Roche/Hitachi Cobas 6000 Chemistry Analyzer (Cobas 6000) is used to measure a wide variety of analytes on various body fluid specimens. It uses an ion specific electrode (ISE) measuring system as well as a photometric measuring system. The ISE system is used in the quantitation of sodium, potassium and chloride. The photometric system can measure colorimetric or nephelometric reactions utilizing end point or kinetic (rate) absorbance measurements. Test ordering on the Cobas 6000 and resulting in the Misys host computer system may be done manually or these tasks may be executed by use of a barcode-based bi-directional interface. The Cobas 6000 can utilize both of these two systems simultaneously.

A. Theory of Operation

Whether directed by the user or by an interfaced order, each specimen is aspirated and delivered to the two different measuring systems. ISE specimens (sodium, potassium, chloride) pass through the electrode measuring system, while photometric specimens are delivered to one of 160 reaction cells, along with up to four reagents. Endpoint or rate reactions occur in the reaction cell with absorbance changes measured using discrete wavelength settings.

Following completion of the reaction, the cell rinse unit washes the reaction cell, and the cell is re-used. All analyses occur at 37°C.

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

The approximate dead volume for the Cobas 6000 microsample cups is 50 uL. It is 50-75 uL for the macro cups.

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 (5 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.

C. Instrument Overview

A general description of the instrument and its components is found in Section A of the Analytics Operator's Manual, Book 1.

The Cobas 6000 has spectrophotometric and ion specific electrode measuring systems. The instrument utilizes reusable optically pure plastic reaction cells that are changed on a quarterly 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 barcoded ID label on them so they can be placed in any position in the appropriate reagent rotor. 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.

Tests can be ordered manually or they can be executed by use of the bi-directional interface connected to the Misys host computer. Similarly, reporting can be achieved through the interface, or by manually keying the results into Misys from the instrument's hard copy printout.

The Misys Method Code for the Cobas 6000 when using the OEM (Online Entry Management) function

D. Instrument Setup

- 1) Log into the Cobas 6000 using assigned username and password.
- 2) Reagents. All reagents used on the Cobas 6000 are stored in a refrigerated reagent compartment. Cholesterol is a one-reagent system. Reagent 1 must be placed in the outer (R1) rotor. All reagents have a unique barcoded identifier. Before starting the analysis sequence check the reagent status on the Cobas 6000 to confirm there is adequate reagent to complete the anticipated test volume for the day. Discard any bottles that have gone empty. Check the volume of the two wash reagents.
- 3) Maintenance. Complete the scheduled daily maintenance as described in the Cobas 6000 general operations protocol.
- 4) Order calibration, if indicated (see Cobas 6000 general operations protocol).
- 5) Order controls. If a calibration was requested, the controls should not be ordered until the calibration report has printed. If the controls are ordered and executed before the calibration prints out, the controls will be measured on the previously stored calibration line.

E. Loading Calibrators and Controls

Each calibrator and control has a defined location on the black calibrator sample 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 racks. A micro- or standard sample cup may be used to hold the calibrators. 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, while in a simple two-point calibration, the standard is only sampled in duplicate). Most calibrations require saline as the “zero” standard. Make sure it is loaded. The sample cups are loaded into the calibrator sample racks like the controls and specimens will be later: seated down into the rack until the flange of the cup is level to the top surface of the rack. If the cup is not seated down far enough, the Cobas 6000 may not be able to detect it through the window in the rack’s mid-section. Additionally, if the sample cup is not fully seated, it could be jarred from the rack during transport on the Cobas 6000.

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

F. Requesting a Calibration: (Operator’s Manual Section B, Book 1)

- 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 methods utilize a two-point calibration. Generally on the

Cobas 6000, 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. The two most common flags found in a failed calibration are SENS and DUP.
 - 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 both reagent bottles, and repeat the calibration.
 - All calibration lines are assayed in duplicate. DUP (duplication error) occurs when the pair of measurements at the zero or measuring point does 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.).
 - Other calibration topics are found in the Operator's Manual, Section B, Book

G. Loading Reagents: (Operator's Manual Section B, Book 1)

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. When a test is highlighted on this list, its reagent bottle locations are shown in the window on the right side of the screen.

There are two concentric reagent carousels: R1 and R2. R1 is the outer ring; R2 is the inner. 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. The two reagent carousel chambers are refrigerated, with a locking lid to ensure constant temperature. There are 48 positions in each rotor. Forty-four of these are available for test reagents, three are reserved for system wash reagents, and one is left open to allow barcode scanning of the inner rotor. All reagent containers have a barcoded ID on their side. This barcode contains lot number, test code, expiration date and available test count information. 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. If the reagents are placed in incorrect rotors, or if the barcode is illegible, the Cobas 6000 will not allow performance of the assay. Reagents may be placed in any position of the appropriate rotor since the barcode directs the Cobas 6000 to the correct rotor location for pipetting. The Cobas 6000 automatically reads the barcodes each time a lid is removed and replaced over the reagent chamber.

H. Loading Specimens: (Operator's Manual Section B, Book 1)

These instructions are provided under the assumption that all sample IDs and test codes will be manually entered by the Cobas 6000 user. The instructions for specimen loading utilizing the bi-directional interface and barcode reader can be found in the separate Cobas 6000: interface protocol.

- 1) Transfer the specimen from its original labeled container to a Cobas 6000 sample cup.
- 2) Place the cup into the next available sample position in a gray, 5-place sample rack.
- 3) With the original container still in hand, enter the desired demographics for the specimen:
 - a. <Workplace>
 - b. <Test Selection>
 - c. In <Type> field select the specimen type (Ser/PI or Urine) from the dropdown menu.
 - d. Enter the specimen ID number in the Sample ID field. <Enter>
 - e. Click or touch screen on the desired tests. Most individual tests are on the Sheet 1 tab. Test panels are on Sheet 2.
 - f. To assign a testing position for the vial, click <Barcode Read Error>.
 - g. Enter Rack No. and Position in the corresponding fields. Note that position 1 on each rack is on the right end of the rack. The gray racks are defined for serum testing; the yellow racks are defined for urine testing.
 - h. .<Add>.
 - i. <OK>.
 - j. <Save>.
- 4) Continue this process until 10 specimens (two racks) have been ordered. Additional specimen loading can occur while the instrument is running.
- 5) If the reagent status is acceptable and the necessary calibrations have been ordered, load the sample racks on to the Cobas 6000. Lift the lid of the sample loading compartment, and place the racks onto track. Since the track (and the slot in the racks) is offset, there is only one way to load them onto the instrument.
- 6) After the racks are loaded close the lid and click <Start>
- 7) The Start Conditions window appears. Click the big <Start> to begin the run.
- 8) The other options on this screen have uses described in the Operator's Manual. The primary field of interest is the Masking field. If any tests are to be turned off, click on Masking, then highlight the tests. Click on Mask to turn the test off. Test masking is primarily done with the HDL (precipitate) method so that it is not executed when the other tests are executed through communication with the host computer system.

- 9) The instrument will go through its homing checks, wash several reaction cells, and then begin delivering the calibrators, controls, specimens and reagents to the reaction cells.
- 10) After this process has begun, additional tests may be ordered by returning to the Test Selection screen. Tests may be added at any time, but the Cobas 6000 will not allow the Start button to be activated if it is flashing. This delay occurs after the last sample rack has entered the sampling chamber. It usually lasts ~ 1 minute.

I. Operation of Assay Procedure 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

After calibration and controls have been measured and evaluated, the test specimens may be loaded onto the Cobas 6000. An abbreviated description of the measurement procedure follows. A more thorough description may be found in the Cobas 6000 general operations protocol.

- 1) If specimens have been frozen, allow them to thaw completely, and then mix well. Serum specimens should not require centrifugation unless they have large amounts of suspended material.
- 2) To order non-barcoded tests on the Cobas 6000:
 - a. <Workplace>
 - b. <Test Selection>
 - c. Enter specimen ID in the Sample ID field, then <Enter>
 - d. Select test HDLC3 by touching the screen or clicking on it with the mouse.
 - e. <Barcode Read Error>
 - f. Enter the rack number and rack position in the Rack No.-Pos. fields.
 - g. <Add>
 - h. <OK>
 - i. <Save>
- 3) Note the order of positions 1-5 in the sample rack: position 1 is on the right and position 5 is on the left. Place the specimen in the rack so that ¼ to 1 inch of the vial is above the sample rack. This allows the Cobas 6000 to detect the presence of the vial in the rack. Orient the vial in the rack so that any barcodes are turned inward, and therefore unreadable. If the testing vials are to be re-capped, arrange the caps so they can be matched up following analysis.

- 4) To order barcoded tests on the Cobas 6000: Follow instructions as in step 3 above, except that the barcode now must face outward so the Cobas 6000 can read it. The barcode must be oriented vertically. No test ordering is required on the instrument. In this case test ordering has occurred in Misys, and a label has been generated for that purpose, or the specimen has a non- Misys barcode label and a user-defined default battery has been installed on the Cobas 6000.
- 5) After the specimens are in place, put the racks onto the loading platform. The racks will only load in one orientation, as the center track is offset. Do not prepare more than three racks at a time, as evaporation could occur while the instrument goes through the sampling process.
- 6) Close the cover on the loading platform.
- 7) On the Cobas 6000 computer terminal, press or click <Start>, then <Start> again.
- 8) Only calibration and control data automatically print out. Patient data hard copies must be requested in <Workplace>, <Data Review>. Highlight the desired records, then <Print>, and <Print> again.
- 9) Non-barcoded records must be manually entered into Misys, a designated spreadsheet, or website.
- 10) Barcoded records are accepted using the OEM program in Misys. The method code for the Cobas 6000 is UR9.

J. Instrument shutdown

After the patient specimens and final controls have been evaluated and accepted, load the green rack (W999) and run it through the instrument. Place three standard sample cups in positions 1, 2 and 3. Fill cup 1 with 1N sodium hydroxide, fill cup 2 with 4N sodium hydroxide, and fill cup 3 with leftover serum. Place it onto the loading platform and press <Start>, and <Start> again. After 18 minutes, the Cobas 6000 comes to Stand-by status. If the green rack is not run, the Cobas 6000 will take at least one hour to come to Stand-by status.

After coming to Stand-by status the data from each day's run is downloaded from the Cobas 6000 computer to a diskette, then to the network folder.

Consult the procedure describing this process for details.

Print all Cobas 6000 test results, and file in chronological order with the other daily printouts.

The Cobas 6000 is turned off each day after all work is complete. The steps are as follows: <Utility>, <Maintenance>, <Nightly Pipe>, <Select>, <Execute>. This shutdown process requires approximately five minutes. The instrument and its computer are automatically turned off. The reagent compartment remains refrigerated.

An automatic timer has been set so that the Cobas 6000 turns on each weekday morning at 0630, automatically performing an air purge, photometer check, and incubator bath exchange during the process. The automatic timer has been set so that the Cobas 6000 remains off during weekends.

Return all leftover controls and calibrators to the refrigerator at the end of the day.

K. Recording of 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>. Make sure the ribbon is in satisfactory condition prior to beginning the day's batch. After evaluating all controls and calibrations, take the hard copy printout to a Misys terminal, and create a worksheet (function MWS) by entering all of the specimen accession numbers in their printout order. Within a given battery of specimens there may be multiple worksheets that have to be created. Enter the results into these worksheets, and then print out the hard copy to check against the instrument printout. The worksheets to be created are defined within the Misys protocols developed for each study. After checking the worksheet result printout, initial the report and place it in the box in room L271.

Treatment of interfaced results is discussed in the separate Cobas 6000 interface protocol.

Assay Performance	Ranges
Analytical Measurement Range	4 - 112 mg/dL
Reportable Range	4 - 224 mg/dL
Limit of Detection (standard 1 + 3SD)	4 mg/dL
Intra-assay %CV (10 within-day replicates at a concentration of 32.0 mg/dL)	0.0%

L. Dilutions

The confirmed analytical measurement range of the assay is 0-121 mg/dL (serum). Specimens exceeding the high limit are automatically diluted (1:2) by the instrument. Results from samples diluted using the rerun function are automatically multiplied by 2. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. For example, to perform a 1:5 dilution, pipette 50 µL of the patient sample into 200 µL of normal saline. Mix thoroughly, perform the assay, and multiply the result by a factor of 5. The maximum allowable manual dilution is 1:5.

M. AMR

The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. CAP LN2 and ABL products are used to validate the HDL analytical measurement range and shipped automatically twice per year. Follow package insert instructions to prepare the samples. Analyze the samples in duplicate, programming in the same manner as patient samples.

Determine the mean value for each level. Results are submitted to CAP electronically by the lead or supervisor and paper copies of the documentation is saved in the CAP binder. CAP reports acceptability limits and linear range shortly after all results are reported.

N. New Lot Verification

Each new reagent lot must be verified for acceptability before being placed into use. Calibration, quality control, and comparison of at least 5 patient samples on the old and new

lots must be performed and found to be within acceptable limits before a new lot can be placed into use.

9. REPORTABLE RANGE OF RESULTS

Roche ranges:

	No risk	Moderate risk	High risk
Female	> 65 mg/dL	45-65 mg/dL	< 45 mg/dL
Males	> 55 mg/dL	35-55 mg/dL	< 35 mg/dL

Advanced Research and Diagnostic Laboratory ranges:

Serum, adult male: reference range >40 mg/dL

Serum, adult female: reference range >50 mg/dL

10. QUALITY CONTROL (QC) PROCEDURES

- A. Normal pooled serum control (CQ). Stable at -80°C for up to 4 years, at refrigerated temperature for up to 1 day and at room temperature for up to 4 hours.

Prepare a 1:2 dilution of the normal pooled serum control with saline. This dilution should be prepared the day specimens are to be run, and may be discarded at the end of the day.

Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed at the end of the shift, with change in reagent, after major maintenance, or as needed for troubleshooting.

If QC values are outside of specified ranges, do the following, in order, until QC is acceptable:

- 1) Repeat the analysis using fresh QC material.
- 2) Perform a calibration.
- 3) Check for system problems.
- 4) Contact Roche Technical Support for assistance and possible service dispatch.

- B. Evaluation of Control Limits

Before the control chart can be used for quality control, it is reviewed to determine that the data have been collected during a stable analytical period. The chart is examined for outliers, for periods of questionable or unstable performance, and for evidence of excessive bias. An outlier will distort the control limits if incorporated into the final calculations. An outlier is considered to be any value of X which falls outside the control limits ($X + 3Sx$) or any value of R which exceeds the control limit for R . These values are eliminated as are values from any questionable period of performance. The values of X , Sx , and the control limits are recalculated and the charts are evaluated again.

The criteria used in the Laboratory were those designed to minimize both bias and variability.

C. Construction of Control Charts

A separate control chart is constructed for each analyte in each control pool. Construct each chart so that plots for X are arranged one above the other on the same sheet of graph paper. Draw the X line across the entire sheet; draw the warning- and control limits parallel to the X line.

Plot the run mean and range values. The chart should be kept current; the values should be plotted after each run. Make liberal use of annotations indicating events that might affect the analyses (personnel changes, reagent problems, changes in instrument components, etc.).

D. Use and Interpretation of Control Charts

Values for X which exceed the 3Sx limit indicate the run is 'out-of-control'. The run must be repeated. Statistically, one in 100 runs can be expected to be 'out-of-control' during normal stable operation. A value exceeding the warning limit, but not the control limit, is interpreted as an indication of possible trouble, but does not necessarily require action. Statistically, about one in 20 values will exceed the warning limits.

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

If control values are out of the acceptable range, recalibration is required. Reanalyze any patient samples after recalibration.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- A. Perform a cell wash daily with SMS/Acid Wash to prevent magnesium hydroxide from being deposited in the cell wash tubing line.

Criterion: Recovery within $\pm 10\%$ of initial value.

- B. Icterus: No significant interference up to an index of 30 for conjugated bilirubin and 70 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 30 mg/dl or 513 $\mu\text{mol/l}$; approximate conjugated bilirubin concentration: 70 mg/dl or 1197 $\mu\text{mol/l}$).

These claims are based on the Glick model. Please refer to further comments below (abnormal liver function).

- C. Hemolysis: No significant interference up to an H index of 1200 (approximate hemoglobin concentration: 1200 mg/dl or 745 $\mu\text{mol/l}$).
- D. Lipemia (Intralipid): No significant interference up to an L index of 1000. No significant interference from native triglycerides up to 1200 mg/dl. There is poor correlation between turbidity and triglycerides concentration.

The claim for lipemia interference is based on the Glick model, which uses Intralipid as an artificial substrate. To date, there is no model available which can mimic interference by triglycerides, as triglyceride levels in patient specimens behave unpredictably, depending on the nature of the esterified fatty acids in the samples. Patient specimens with elevated

triglyceride levels are very often lipemic. Therefore customers cannot verify interference by triglycerides in patient specimens.

- E. Elevated concentrations of free fatty acids and denatured lipoproteins may cause falsely elevated HDL-cholesterol results.
- F. Ascorbic acid up to 50 mg/dl does not interfere.
- G. In rare cases, elevated immunoglobulin concentrations can lead to falsely increased HDL-cholesterol results. Abnormal liver function affects lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-C plus result is significantly negatively biased versus the DCM (designated comparison method) result. Of 20 commonly used drugs tested in vitro, none interfered with the assay.

13. REFERENCE RANGES (NORMAL VALUES)

Linear range of the method: 0-120 mg/dL. Specimens exceeding the high limit are automatically diluted (net 1:2) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.

Analytical Measurement Range: 0-120 mg/dL Clinically Reportable Range: 3-200 mg/dL

14. CRITICAL CALL RESULTS ("PANIC VALUES")

The critical call results for this test is ≤ 40 mg/dL

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Samples are received frozen and stored at -80°C until testing is performed. Upon completion of analysis, specimens are stored for 1 week. NHANES specimens are frozen at -70°C and discarded after 1 year.

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

Samples are held at -80°C in the freezer. Samples are held at -80°C in the freezer. If a problem occurs and this freezer begins to warm samples are transferred to the research freezers. A service call is placed to repair the freezer.

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

Critical action values are called into the Medical Officer for cholesterol values.

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

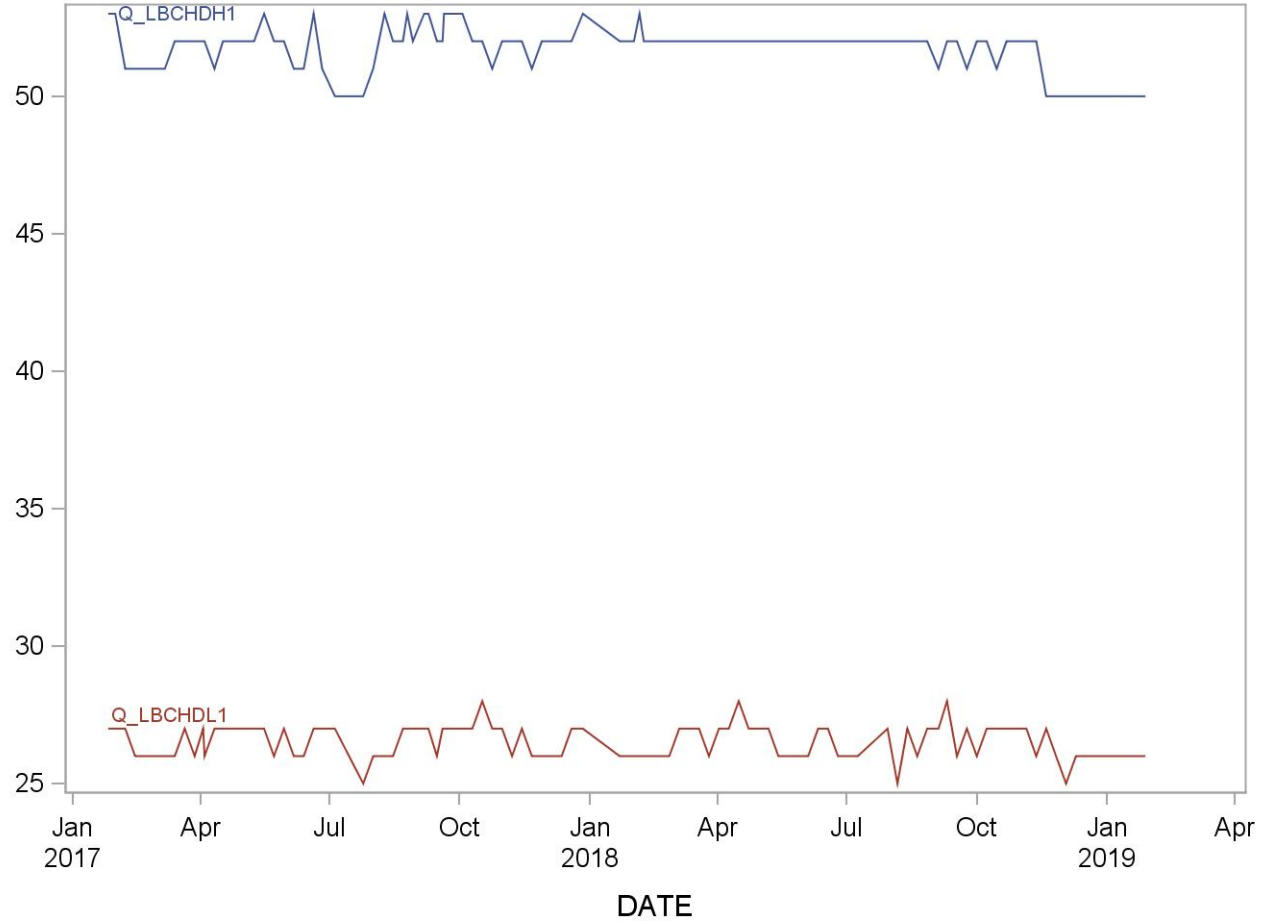
All shipments are recorded on the NHANES Shipping Log upon receipt. Actions taken during the course of analysis, result reporting, and specimen retention are also recorded on the log

19. SUMMARY STATISTICS and QC GRAPHS

See following pages.

Summary Statistics and QC Chart for Direct HDL-Cholesterol (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Q_LBCHDH1	99	26JAN17	28JAN19	51.8	0.8	1.5
Q_LBCHDL1	99	26JAN17	28JAN19	26.5	0.6	2.3



REFERENCES

- Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.
- Roche Precipath U Plus package insert, Version 2011-04, V4. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
- Roche HDLC3 reagent package insert. Version 2012-04, V7. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
- Roche C.F.A.S. Lipids calibrator package insert. Version 2013-07, V6. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.