

Laboratory Procedure Manual

Analyte: **Apolipoprotein B**

Matrix: **Serum**

Method: **Turbidimetric Assay on Roche
Cobas® 6000**

Method No.:

Revised:

As performed by: **University of Minnesota – Advanced
Research Diagnostics Laboratory
(ARDL)**

Contact: **Dr. Anthony Killeen, MD, PhD
University of Minnesota Medical Center
Fairview-University Medical Center
University Campus
Minneapolis, Minnesota**

Important Information for Users

University of Minnesota – Advanced Research Diagnostics Laboratory (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 in the following table:

Data File Name	Variable Name	SAS Label
APOB_I	LBXAPB	Apolipoprotein B (mg/dL)
	LBDAPBSI	Apolipoprotein B (g/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A. Clinical Relevance

Apolipoproteins are the protein constituents of the lipoproteins. Apolipoprotein B (Apo B) is the major protein component of low-density lipoprotein (LDL). About one-third of the LDL particles provide cholesterol to peripheral cells. The other two-thirds are metabolized by the liver. LDL-uptake in all of these cells occurs via LDL receptors. Apo B levels increase in hypercholesterolemia, pregnancy, LDL receptor defects, bile obstruction and nephrotic syndrome. Apo B levels decrease in liver disease, sepsis and estrogen administration.

The combined measurement of apolipoprotein A-1 (Apo A1, present in HDL) and Apo B and the calculation of the Apo B:Apo A1 ratio can reflect a lipid metabolism disorder and the risk of developing atherosclerosis or coronary heart disease. A high level of Apo A-1 and a low level of Apo B correlate best with a low risk for these diseases.

This method utilizes anti-Apo B antibodies that react with the antigen in the sample to form antigen/antibody complexes which, after agglutination, are measured turbidimetrically. The primary measuring wavelength is 340 nm; the secondary wavelength is 700 nm. This is a 2-point, endpoint reaction that is specific for Apo B.

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 using a wide variety of tests. The Cobas 6000 analyzer series is optimized for workloads using a combination of photometric and ion-selective electrode (ISE) determinations (c501 module), and electrochemiluminescence (ECL) signal 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 end 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 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 of 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.

To access the spreadsheet click on Computer → S drive → ARDL Shared → NHANES Lipids → desired spreadsheet.

Choose the file named with the corresponding box number.

Enter the analysis date, run number, technologist's initials, result, and result comment code.

The spreadsheet will be sent electronically by the contact person.

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

Serum is the only acceptable specimen type for the procedure. Serum or lithium heparin, K2-EDTA plasma is acceptable. The serum is stable for eight days at 4°C, and longer at -70°C.

Serum specimens that have been frozen are sometimes prone to excessive precipitate formation. These specimens should be centrifuged to remove these particulates. Excessive particulate matter may also 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.

The minimum volume is 100 µL in a sample cup, or 2 mL microtube (9 µL for test; remainder for dead volume). Dead volume is not recoverable if sample is transferred into a sample cup.

Serum or plasma is stable for one day at 15-25°C, eight days at 2-8°C, two months at (-15 to -25)°C, and longer at -70°C. Centrifuge specimens containing precipitates prior to analysis.

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

Not applicable for this procedure.

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

A. Equipment and Supplies

1. Roche/Hitachi Cobas 6000 Analyzer. Roche Diagnostics, 9115 Hague Road, P.O. Box 50457, Indianapolis, IN, USA 46250-0446.
2. Purified water supply. The Cobas 6000 need 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.
3. Cobas 6000 system reagents.
 - a. 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.

- b. 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.
- c. 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.
- d. 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.
- e. 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.
- f. 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.
- g. 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.
- h. 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.

- i. 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.
- j. Sample cups (micro). Roche product #05085713.
- k. Sample cups (standard). Roche product #10394246.
- l. Printer paper, 8.5 x 11 inch. Various sources including Bose Multipurpose Paper.
- m. Printer cartridge. HP Laserjet CE505A, black. Hewlett-Packard Company.
- n. Reagents and calibrators. See specific assay procedures.
- o. 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.

C. Preparation of Calibrator

1. Roche C.F.A.S. Lipids Calibrator, Cat. No. 12172623160: A lyophilized calibrator based on human serum. To prepare, carefully open one bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam. Calibrator is stable when unopened and stored at 2-8°C until the expiration date on the bottle. Once opened, the calibrator set is stable for 5 days when stored at 2-8°C and 4 weeks at -20°C (when frozen once).
2. Traceability: This method has been standardized against the IFCC SP3-07 reference standard (WHO-IRP October 1992).
3. Calibration frequency: The Cobas 6000 will automatically perform a six-point calibration (H₂O + five dilutions of the calibrator) when there is a reagent lot number change. No other auto-calibrations are defined for the assay. The Cobas 6000 will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.
4. Manual calibration should be performed if:
 - a. A reagent lot change has not occurred in the past 6 months

- b. After major service or repairs
 - c. As needed for troubleshooting
5. If calibration fails perform the following corrective action steps in sequence:
 - a. Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation and storage conditions.
 - b. Repeat calibration with new calibrator.
 - c. Repeat calibration with new reagent and new calibrator
 - d. If successful calibration is not achieved, discontinue testing and notify the supervisor.

D. Preparation of Quality Control

1. 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.
2. Roche Precipath U Plus Control (catalog #12149443160). Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at 2-8°C. To prepare, open bottle 1 and pipette in exactly 3.0 mL of diluent (bottle 2). Dissolve by gentle swirling for 30 minutes. Prepared control is stable for 12 hours at room temperature, 5 days at 2-8°C, and one month at -20°C (when frozen once).
3. 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.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration:

To Calibrate:

- a. At the home screen, click or touch screen on <Calibration> tab.
- b. 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.
- c. Click or touch screen on the tests to be calibrated.

- d. 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.
 - e. 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>.
 - f. Click or touch screen on <Save>.
 - g. Failed calibrations will generate an error message by the Cobas 6000.
 - h. For the c501 module, the two most common flags found in a failed calibration are SENS and DUP
 - i. 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.
 - ii. All calibrators 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.).
- B. For the e601 module, the most common flags found 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 aliquotted, 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, and Chapter 12.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

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. These instructions will attempt to cover these scenarios, but there may be nuances to certain studies and their associated testing schemes that the user must be aware.

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.

Manual test ordering:

1. If the original vial is not compatible with the Cobas sampling system, transfer the specimen from its original labeled container to a hand-labeled sample cup.
2. Place the cup into the next available sample position in a gray, 5-place sample rack.
3. 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.
 - 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 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.
 6. After the racks are loaded, return the tray to the loading stations, 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 the Start 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 T-Mask to turn the test off, or click on P-Mask to turn the test off for patient testing but to allow calibration and quality control. Test masking is primarily done with the HDL (precipitate) method so that it is not executed when the other tests are performed through communication with the host computer system.
 9. 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.
 10. After this process has begun, additional tests may be ordered by returning to the Test Selection screen. Tests may be added anytime, 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, unless there is a backup in the holding carousel.

Interfaced test ordering:

1. 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.

2. The desired tests are ordered in the host computer system, STARLIMS.
3. A container ID (CID) is generated by the system, and a label is produced with the barcoded CID on it.
4. This label is affixed to a vial that will be used for direct sampling on the Cobas 6000.
5. After arrival of the specimen at the analyzer, and following mixing and/or centrifugation, the labeled vial is placed into a proper rack.
6. 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.
7. The barcode must face out through the groove in the rack, and the vial must be positioned so it is high enough in the rack to be seen by the location detector, but not so high that it might be ejected from the rack while being transported through the instrument.
8. 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.
9. After the racks are loaded, return the tray to the loading stations, close the lid and click <Start>.
10. The Start Conditions window appears. Click the big <Start> to begin the run.
11. The other options on the Start 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 T-Mask to turn the test off, or click on P-Mask to turn the test off for patient testing but to allow calibration and quality control. Test masking is primarily done with the HDL (precipitate) method so that it is not executed when the other tests are performed through communication with the host computer system.
12. 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.
13. 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 flashing. This delay occurs after the last sample rack has

entered the sampling chamber. It usually lasts ~ 1 minute, unless there is a backup in the holding carousel.

Results:

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.

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. 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-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.

9. REPORTABLE RANGE OF RESULTS:

Analytical Measurement Range (AMR)*	20-400 mg/dL
Reportable Range	20-400 mg/dL
Limit of Detection (LOD) (standard 1 + 3 SD)	3 mg/dL
Intra-assay %CV (10 within-day replicates at a concentration of 63 mg/dL)	2.0%
Intra-assay %CV (10 within-day replicates at a concentration of 160 mg/dL)	0.6%
Inter-assay %CV (22 between-day replicates at a concentration of 35 mg/dL)	2.5%
Inter-assay %CV (23 between-day replicates at a concentration of 84 mg/dL)	1.6%

Dilutions

The confirmed analytical measurement range of the assay is 20 to 400 mg/dL (serum). Specimens exceeding the high limit are not diluted by the instrument; they are reported as >400 mg/dL.

*The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. Use a patient specimen with an

elevated level of Apo B, and make serial dilutions to cover the measuring range. Analyze samples in duplicate.

10. QUALITY CONTROL (QC) PROCEDURES

1. 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.
2. Roche Precipath U Plus Control (catalog #12149443160). Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at 2-8°C. To prepare, open bottle 1 and pipette in exactly 3.0 mL of diluent (bottle 2). Dissolve by gentle swirling for 30 minutes. Prepared control is stable for 12 hours at room temperature, 5 days at 2-8°C, and one month at -20°C (when frozen once).

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.

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

Roche C.F.A.S. Lipids Calibrator, Cat. No. 12172623160: A lyophilized calibrator based on human serum. To prepare, carefully open one bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam. Calibrator is stable when unopened and stored at 2-8°C until the expiration date on the bottle. Once opened, the calibrator set is stable for 5 days when stored at 2-8°C and 4 weeks at -20°C (when frozen once).

Traceability: This method has been standardized against the IFCC SP3-07 reference standard (WHO-IRP October 1992).

Calibration frequency: The Cobas 6000 will automatically perform a six-point calibration (H₂O + five dilutions of the calibrator) when there is a reagent lot number change. No other auto-calibrations are defined for the assay. The Cobas 6000 will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Manual calibration should be performed if:

- A reagent lot change has not occurred in the past 6 months
- After major service or repairs
- As needed for troubleshooting

If calibration fails perform the following corrective action steps in sequence:

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation and storage conditions.
- Repeat calibration with new calibrator.
- Repeat calibration with new reagent and new calibrator

If successful calibration is not achieved, discontinue testing and notify the supervisor.

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.

Contact Roche Technical Support for assistance and possible service dispatch. Phone: 1-800-428-2336; account number: #####.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- Icteric index <60: no interference.
- Hemolytic index <1000: no interference.
- Lipemic index <1000: no interference.
- Drugs (therapeutic concentrations of commonly used drug panels): no interference.
- In very rare cases, gammopathy, in particular IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.
- Rheumatoid factors <1200: no interference.
- High dose hook-effect: No false result occurs up to an Apo B concentration of 900 mg/dL.

13. REFERENCE RANGES (NORMAL VALUES)

The Apo B reference ranges provided by Roche:

Serum, adult male: 66-133 mg/dL

Serum, adult female: 60-117 mg/dL

Apolipoprotein B is the main protein component of LDL and accounts for approximately 95% of the total protein content of LDL. Apo B is necessary for the reaction with LDL receptors in the liver and on cell walls, and is thus involved in transporting cholesterol from the liver to the vessel cell. Elevated levels of Apo B are frequently found in patients with atherosclerotic vascular changes and are a risk factor for atherosclerosis.

Several studies have shown that the assay of apolipoprotein B is helpful in assessing the risk of atherosclerosis and has greater prognostic power than the sole determination of HDL and LDL cholesterol.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

No critical action value(s) exist for apolipoprotein B.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

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

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

Samples are held at -80 in the freezer in 1379. If a problem occurs and this freezer begins to warm. Samples are transferred to the research freezers located in 1358. A service call is placed to repair the freezer in 1379. A loaner freezer is requested for each service repair that removes the freezer from 1379 for any period greater than 1 day.

No alternate test site has been identified. As far as downtime for equipment repairs, the 21 day turnaround time as established in the contract, has always been sufficient enough to allow the repair to occur prior to the deadline for sample analysis. If the repair could not be accomplished in the time frame allowed we will discuss the three options available to us with the project officer. One option is to wait until the repair is made if the proposed repair date is agreeable to the project officer. The second option is would be to use the NWRL since it is the IFCC Apolipoprotein reference laboratory.

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

NHANES results are entered unto a spreadsheet provided electronically.

To access the spreadsheet click on Computer → S drive → ARDL Shared → NHANES Lipids → desired spreadsheet.

Choose the file named with the corresponding box number.

Enter the analysis date, run number, technologist's initials, and result comment code.

The spreadsheet will be sent electronically by the contact person.

Early Reporting Results for NHANES:

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

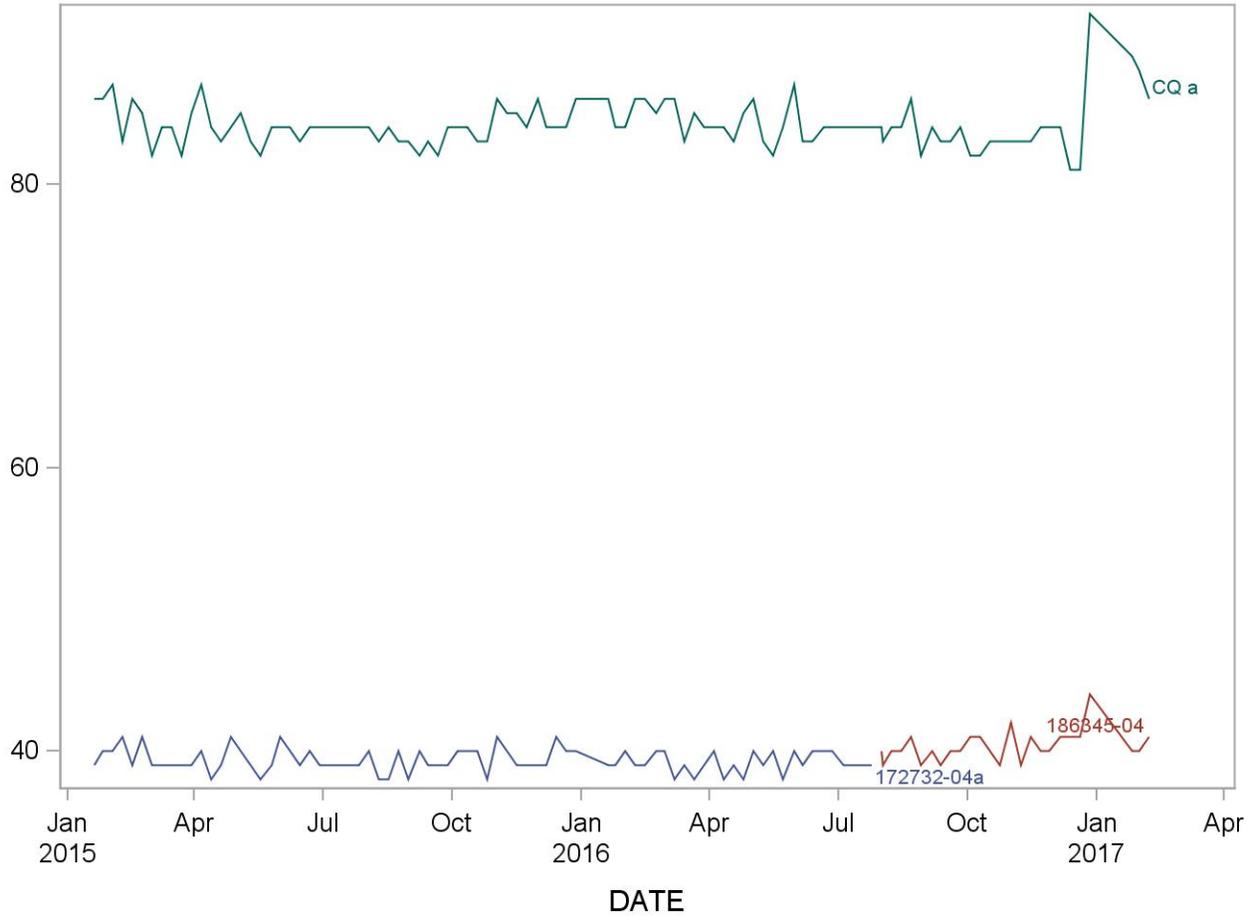
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

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

2015-2016 Summary Statistics and QC Chart for Apolipoprotein (B) (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
172732-04a	74	20JAN15	25JUL16	39.4	0.8	2.1
CQ a	100	20JAN15	07FEB17	84.2	1.7	2.0
186345-04	26	01AUG16	07FEB17	40.3	1.1	2.7



REFERENCES

- Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.
- Roche APOBT reagent package insert. Version 2012-02, V8. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
- Roche CA2 reagent package insert. Version 2012-08, V2. 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.
- Roche Precipath U Plus package insert, Version 2011-04, V4. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.