

Laboratory Procedure Manual

Analyte: **Apolipoprotein B**

Matrix: **Serum**

Method: **Siemens ProSpec Analyzer**

As performed by: Collaborative Studies Clinical Laboratory

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Important Information for Users

The Fairview -University Medical Center 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
APOB_H	LBXAPB	Apolipoprotein B (mg/dL)
	LBDAPBSI	Apolipoprotein B (g/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A. Clinical Relevance

A solution of rabbit-derived anti-Apo B antibody is incubated with the specimen. An immune complex forms between the antibody and the Apo B in the specimen, resulting in an increase in light scatter. The intensity of the scattered light is directly proportional to the concentration of Apo B in the specimen. The Apo B concentration of the test specimen is determined by comparing its light scatter to that observed using known standards in a stored calibration curve.

Apolipoprotein B is the main protein component of LDL cholesterol, comprising 95 per cent of its total protein content. Apo B is necessary for the reaction with LDL receptors in the liver and on cell walls, so it is involved in transporting cholesterol from the liver to the vessel cells. Elevated levels of Apo B are frequently found in patients with atherosclerotic vessel changes and are a risk factor for atherosclerosis. Some studies have shown that the quotient of Apo B/Apo A provides a powerful parameter for estimating this risk: the higher the ratio, the greater the risk of atherosclerotic development.

B. Principle

A solution of rabbit-derived anti-Apo B antibody is incubated with the specimen. An immune complex forms between the antibody and the Apo B in the specimen, resulting in an increase in light scatter. The intensity of the scattered light is directly proportional to the concentration of Apo B in the specimen. The Apo B concentration of the test specimen is determined by comparing its light scatter to that observed using known standards in a stored calibration curve.

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. Anticoagulants are unacceptable. The serum is stable for eight 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 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 ProSpec samples 30 uL. The sample dead volume when sampling from 2 mL Sarstedt vials in the Eppendorf rack is approximately 75 uL. The sample dead volume when sampling from a 10 x 75 mm glass test tube is approximately 250 uL.

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

Not applicable for this procedure.

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

A. Equipment and Supplies

1. ProSpec Nephelometer
Siemens GMBH. Marburg, Germany D-35041.
2. ProSpec Parts and Supplies
 - a) N Reaction Buffer. Siemens product #OUMS 65 (5 L bottle). A solution of polyethylene glycol and sodium chloride (11.6 g/L) in phosphate buffer (0.05 mol/L). Contains sodium azide (<1 g/L). Store at room temperature. Stable until expiration date on bottle; stable for six weeks after opening.
 - b) N Diluent. Dade Behring product #OUMT 65 (5 L bottle). Phosphate buffered saline with sodium azide (<1 g/L). Store at room temperature. Stable until expiration date on bottle.
 - c) N Cuvette segments for BN ProSpec. Siemens product #OVLK 31 (300 segments of 9 cuvettes each). Cuvettes are a single-use item, and must be manually changed by the operator.
 - d) N Predilution wells. Siemens product #OVIC 11 (1100 segments of 6 wells each). Used for automatic dilutions prepared by the ProSpec.
 - e) N Evaporation caps for reagent vials (5 mL). Siemens product #OVLC 31 (100 pieces). The caps are placed directly on reagent bottles stored in the refrigerated reagent chamber. The design allows for sampling by the instrument, but prevents evaporation of the reagent.
 - f) Disposable glass culture tubes, 10 x 75 mm. Cardinal Health cat. no. T1290-2. Specimens are transferred into these tubes prior to instrument sampling.
3. Pipets
 - a) Adjustable, 20-200 μ L and 100-1000 μ L.

B. Reagents

1. N Antiserum to Human Apolipoprotein B Kit
 - a) Siemens GMBH. Marburg, Germany D-35041. Product #OSAN 15. Store at 2 to 8°C until put into use. The kit is stable until the expiration date noted on the box. Once opened, the reagent is stable for four weeks if stored, with its evaporation cap in place, in the ProSpec's refrigerated reagent chamber. The kit contains 5 mL of reagent. This is sufficient for approximately 125 tests. The following component is included:

- 1) N Antiserum to Human Apolipoprotein B, 125 tests, 5 mL bottle. Liquid anti-human Apo B serum obtained from rabbits immunized with LDL isolated from human sera. The titer of antibody is determined by radial immunodiffusion, and it is printed on the reagent bottle label. The titer indicates the quantity of antigen (in mg) which will be precipitated in an agarose gel by 1 mL of the antiserum.

Do not interchange components from different kit lots.

2. N Supplementary Reagent/Precipitation Kit

- a) Siemens GMBH. Marburg, Germany D-35041. Product #OUMU 15. Store at 2 to 8°C until put into use. The kit is stable until the expiration date noted on the box. Once opened, the reagent is stable for four weeks if stored, with its evaporation cap in place, in the ProSpec's refrigerated reagent chamber. The kit contains 5 mL of reagent. This is sufficient for approximately 500 tests. The following component is included:
- b) N Supplementary Reagent/Precipitation, 500 tests, 5 mL bottle. A solution of phosphate buffer, sodium chloride and Thesit®. See package insert for concentrations. This reagent is required when using the Apo B kit described above. A change in the lot number of this supplementary reagent does not require re-calibration of the ProSpec.

3. Milli-Q water

1. Milli-Q is the trade name of the water system purchased from the Millipore Corporation. Milli-Q water is deionized water treated with activated carbon and deionization cartridges and filtered to remove microorganisms larger than 0.22 micrometers. This meets CAP Class I water requirements.

C. Preparation of Calibrator

1. N Apolipoprotein Standard Serum
Siemens product #OUPG 09 (3 x 0.5 mL).
Lyophilized product prepared from human serum. Store at 2 to 8°C until put into use. Dilute standard in 0.5 mL of Milli-Q water, and let stand at room temperature. Invert gently to mix while avoiding foam formation. The Apo B concentration in the standard will vary with lot. Siemens provides periodic calibrator lot and concentration updates on compact disk. When these parameters are read into the ProSpec system, it is only necessary for the instrument to read the calibrator's barcode to determine its Apo B concentration. The reference line is valid until controls demonstrate drift, the reagent lot changes, or the calibrator lot changes. At these junctures, re-calibration must be performed. If capped and refrigerated, the diluted standard is stable for eight days following preparation when stored at 2 to 8°C.

D. Preparation of Quality Control

1. There are two levels of controls routinely assayed. The first is a pooled human serum collected from normal donors. It is stored at -70°C. The second level of control is a commercially available lyophilized product. Consult posted information for current manufacturer, lot numbers and acceptable ranges. The controls are assayed on the BN ProSpec as specimen unknowns, ordered like a routine test specimen. All values are plotted on the spreadsheet located ProSpec folder within the Daily QC Tally folder in the ARDL share drive. Review Levey-Jennings plots and acceptability criteria for each quality control result daily.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration:

To Calibrate:

- a) Load the ProSpec with reagent: <Loading [F4]> <Reagents [F8]> Click on an existing reagent position <Access [Shift+F6]>. Answer OK to the prompt, and the ProSpec positions the selected reagent under the access door. Open the instrument's right cover and the reagent door. Note: Never open either of the ProSpec's covers without requesting, then being granted access by the computer system. If this step is not followed the instrument may have to be reset. The reagent rotor may be manually rotated. Load the reagent into position 1 or 3 in any of the white reagent wedges. The bottle barcode must face out through the window of the wedge in order for the ProSpec to register it. The yellow wedges are for controls only. Close the covers after loading, and answer the prompts on the computer to commence registration.
- b) Load the ProSpec with calibrator. Remove the cap, and then place a bottle of N Apolipoprotein Standard Serum in any position on the calibrator segment (#2001). Turn the bottle so its barcode faces out through the gap in the holder.
- c) Click on <System> <Replace Sample>. Accept the prompt, and then open the left cover of the instrument. Place the calibrator segment in any of the three locations on the sample rotor.
- d) Close the left door and answer the computer prompts. The ProSpec is now ready for calibration.
- e) Click on <Calibration [F5]> at the top of the screen.
- f) In the Calibration window, select test <APOB> in the Assays box.
- g) In the Reagents box, select the appropriate reagent lot number requiring calibration.
- h) If the reagent lot has never been calibrated, click on the <Measure> icon on the left of the screen.
- i) If the reagent lot has been previously calibrated, click on the <Show Curve> icon on the left of the screen. When the existing curve is displayed, click on the <Repeat> icon on the left of the screen. The ProSpec will store up to two calibrations for three reagent lot numbers. If three lot numbers are already present, delete the oldest one before requesting the calibration on the new lot. If this is not done, the ProSpec

- will not execute the new calibration. The calibration process begins automatically, and requires approximately 12 minutes.
- j) The ProSpec prepares the serial standard dilutions required to construct a complete standard line. The instrument will automatically accept the line if it meets established limits. Print a copy of the calibration curve by following steps e, f, g, and i above. Click on the printer icon to create a hard copy. Keep this copy in the logbook along with a printout of the day's results.

B. Calibration Verification:

Refer to Calibration Verification guidelines in Standard Operating Procedures.
Reagent and Calibrator lot Verification

All reagent and calibrator lots are validated with 5-10 samples run with both the old calibrator /reagent run values vs. the newly calibrated channel or new reagent lot. All values must be within 5% of the older lot analysis. If the values are greater than 5% the lot is rejected for use.

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

1. If specimens have been previously frozen, allow them to thaw completely, then mix well. Remove any particulates as described in the Specimen section above.
2. The ProSpec does not have a set time period for mandatory recalibration. Calibration is performed if control values deviate, or if the reagent lot number changes. To calibrate:
 - a) Load the ProSpec with reagent: <Loading [F4]> <Reagents [F8]> Click on an existing reagent position <Access [Shift+F6]>. Answer OK to the prompt, and the ProSpec positions the selected reagent under the access door. Open the instrument's right cover and the reagent door. **Note: Never open either of the ProSpec's covers without requesting, then being granted access by the computer system. If this step is not followed the instrument may have to be reset.** The reagent rotor may be manually rotated. Load the reagent into position 1 or 3 in any of the white reagent wedges. The bottle barcode must face out through the window of the wedge in order for the ProSpec to register it. The yellow wedges are for controls only. Close the covers after loading, and answer the prompts on the computer to commence registration.
 - b) Load the ProSpec with calibrator. Remove the cap, then place a bottle of N Apolipoprotein Standard Serum in any position on the calibrator segment (#2001). Turn the bottle so its barcode faces out through the gap in the holder.
 - c) Click on <System> <Replace Sample>. Accept the prompt, then open the left cover of the instrument. Place the calibrator segment in any of the three locations on the sample rotor.
 - d) Close the left door and answer the computer prompts. The ProSpec is now ready for calibration.

- e) Click on <Calibration [F5]> at the top of the screen.
 - f) In the Calibration window, select test <APOB> in the Assays box.
 - g) In the Reagents box, select the appropriate reagent lot number requiring calibration.
 - h) If the reagent lot has never been calibrated, click on the <Measure> icon on the left of the screen.
 - i) If the reagent lot has been previously calibrated, click on the <Show Curve> icon on the left of the screen. When the existing curve is displayed, click on the <Repeat> icon on the left of the screen. The ProSpec will store up to two calibrations for three reagent lot numbers. If three lot numbers are already present, delete the oldest one before requesting the calibration on the new lot. If this is not done, the ProSpec will not execute the new calibration. The calibration process begins automatically, and requires approximately 12 minutes.
 - j) The ProSpec prepares the serial standard dilutions required to construct a complete standard line. The instrument will automatically accept the line if it meets established limits. Print a copy of the calibration curve by following steps e, f, g, and i above. Click on the printer icon to create a hard copy. Keep this copy in the logbook along with a printout of the day's results.
3. If calibration is not required, or if the calibration has been successfully completed, the next step is to assay controls. Controls on the ProSpec are assayed like specimen samples:
- a. Click on <Lab Journal [F3]> at the top of the screen.
 - b. Select <New [F7]> from left-side menu.
 - c. Key in the control (or specimen) ID in the Sample Data field. Use the <TAB> key to move between fields.
 - d. In the Assays field select <APOB> from the dropdown menu.
 - e. Select <Apply> to accept.
4. After the controls are ordered, load the specimens onto the instrument. Before loading, clear any existing specimen assignments from all sample segments:
- a. At top of screen select <Loading [F4]>.
 - b. In the Segment ID dropdown field select the sample segment to be cleared (010108, 010207, 010306, 610100, 610209, or 610308). Note: the labels on the sample segments do not include the final two digits.
 - c. Double right-click on any assigned cell.
 - d. Answer <YES> to the dialog box that appears. This will clear all specimens from this segment.
 - e. Click on <Apply [Shift+F10]> in the lower left screen corner.
 - f. Exit the Loading window by choosing <System> at the top of the screen. This step clears the IDs that were just removed from the segment from the pending test list.
 - g. Again select <Loading [F4]>.
 - h. Select the Segment ID from the drop down menu in the center of the screen.
 - i. From the ID box at screen right, left-click-hold on the desired ID. Drag the ID to the segment cell that will hold the specimen. Continue for all

IDs. After all specimens are transferred or if additional segments are needed, click on <Apply>. If additional specimens are to be loaded, then select the next segment to load. Continue this process for the remaining IDs.

5. With the loading window still on the screen, or after printing out the loading window, begin transferring the specimens to the sample segment.
6. If using sample segments 010108, 010207 or 010306 transfer 300 uL of control or specimen to a 10x75 mm disposable glass tube, then place the tube in the correct location on the sample segment. The sample segments have their background barcode blocked by a piece of paper so that the instrument will not detect the barcode through the glass tube, and interpret that as a missing specimen. Do not remove this paper.
7. If using sample segments 610100, 610209 or 610308 place a 2 mL Sarstedt vial directly into the rack. If the specimen was originally provided in this type of vial place it directly into the rack and turn any barcode labels inward so they are not read by the instrument. Alternatively, the specimen may be transferred to an empty Sarstedt vial for testing as with the 10 x 75 mm glass tubes in step 6. In either case, the volume of specimen in the Sarstedt vial must not exceed 1 mL as this causes sampling interruption on the ProSpec.
8. After all of the specimens and controls have been loaded onto sample segments, place the segments onto the sample rotor. Access must be requested by selecting <System>, then <Replace Samples>. Answer <OK> to the prompt, then wait for access clearance.
9. Lift the left door, and place the sample segments into the rotor brackets. The segments may be loaded in any sequence. Close the left door. When prompted, click on <Close [Shift+F12]>. Answer <OK> at the next prompt.
10. The ProSpec now automatically begins sampling, diluting and measuring. There is no "Start" button on the instrument. The System screen will show the amount of time remaining in the programmed run, and it will alert the operator to any error messages. Common problems and their solutions are found in the Instruction Manual, Chapter 14.
11. The run is complete when the instrument functions have ceased, and the Walk Away Time on the System screen reads 0 minutes. Remove specimens from the instrument by requesting access to the sample rotor: <System>, <Replace Samples>. Answer the prompts as above when loading, then remove the segments and close the door. Before discarding the tubes into a biohazard waste container, review results as described in the next section.
12. Before preparing another batch of specimens, check reagent volume and dilution cup and cuvette availability on the instrument. Replenish or replace, as necessary.

13. There is no shutdown procedure for the ProSpec. Remove all completed specimens, empty reagent bottles and used dilution cups and reaction cuvettes at the end of the day.

E. Results

1. Results will not print out automatically. At the conclusion of the run, review the results by selecting <Lab Journal [F3]>. Results may be printed from this screen by highlighting the results to print, then selecting the Print icon.
2. The measurable reporting on a routinely (1:20) diluted specimen is 25-400 mg/dL. This range may vary slightly depending upon calibrator lot number. Results exceeding the upper concentration limit for a given dilution will be automatically diluted and repeated by the ProSpec. Results reading less than the lowest limit will print out as such.
3. Results are reported as a whole number in mg/dL. Results reading <25 mg/dL are reported as such.

9. REPORTABLE RANGE OF RESULTS:

Analytical Measurement Range: 25-400 mg/dL

Clinically Reportable Range: 25-1000 mg/dL

10. QUALITY CONTROL (QC) PROCEDURES

There are two levels of controls routinely assayed. The first is a pooled human serum collected from normal donors. It is stored at -70°C. The second level of control is a commercially available lyophilized product. Consult posted information for current manufacturer, lot numbers and acceptable ranges. The controls are assayed on the BN ProSpec as specimen unknowns, ordered like a routine test specimen. All values are plotted on the spreadsheet located ProSpec folder within the Daily QC Tally folder in the ARDL share drive. Review Levey-Jennings plots and acceptability criteria for each quality control result daily.

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

Hold one specimen from each day's batch as a between-batch duplicate. Record the result on the vial label, and return it to the pending rack. Re-assay it in the next batch. The difference in the results must be within the current posted QC duplicate limit. The NHANES duplicate protocol is sufficient for this purpose.

Re-calibrate the ProSpec when the Apo B antibody kit lot changes. Recalibration is not necessary if only the supplementary reagent lot changes. Assay at least five specimens on the old lot and on the new lot. Each of their differences must be within the current posted QC duplicate limit.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Turbidity and particles in the sample can interfere with the test. Particles resulting from incomplete coagulation or denaturation of proteins should be removed prior to assay by centrifugation.

In isolated cases excessive concentrations of triglycerides or hyperlipidemia samples may disturb the Apo B assay. In such cases retesting the sample at a higher dilution can reduce the effect of the disturbance.

13. REFERENCE RANGES (NORMAL VALUES)

The Apo B reference ranges provided by Siemens are:

Women:	55-125 mg/dL
Men:	55-140 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 exists for Apo 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

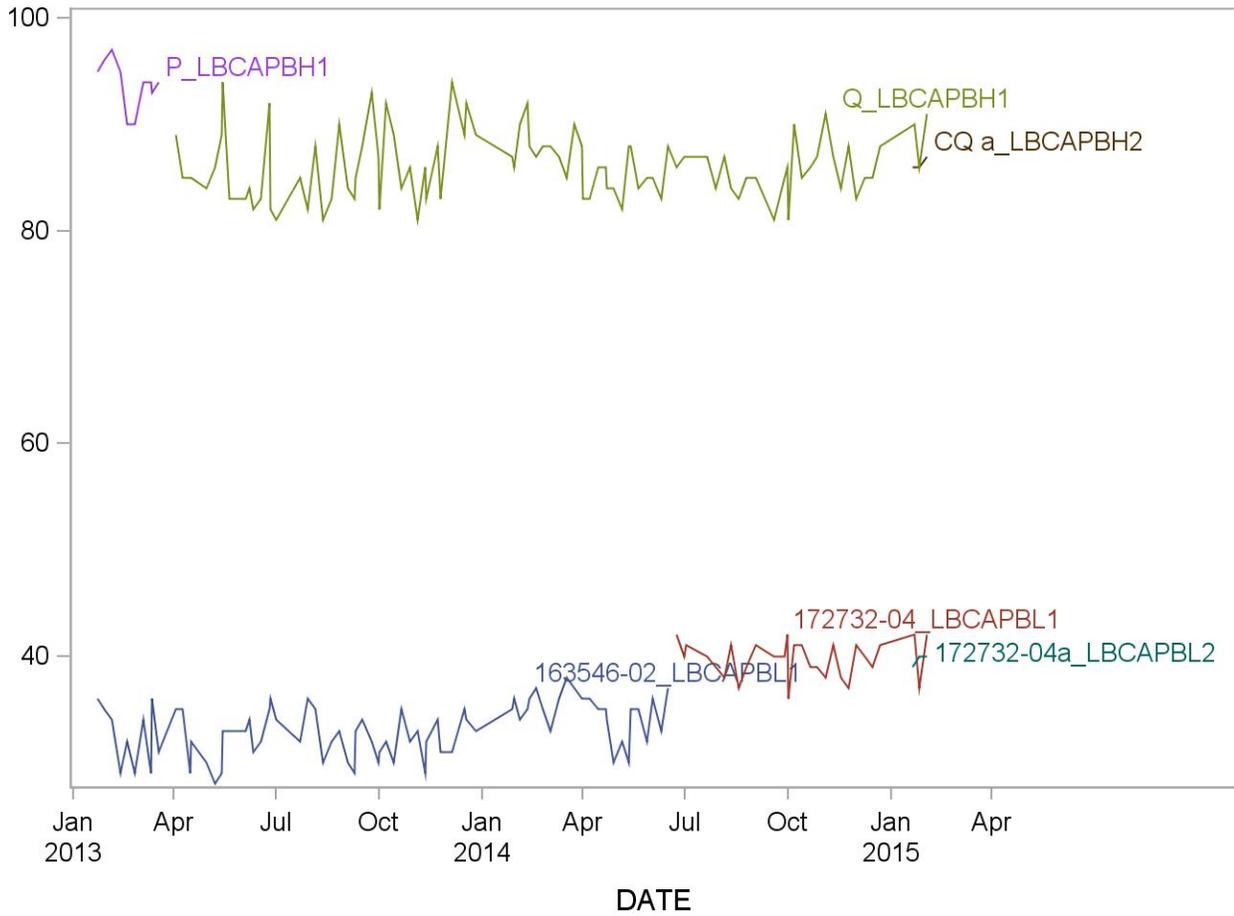
All shipments are recorded on the NHANES Shipping Log upon receipt. Actions taken during the course of analysis, result reporting, and specimen retention are also recorded on the log.

19. SUMMARY STATISTICS AND QC GRAPHS

See the following page.

2013-2014 Summary Statistics and QC Chart for Apolipoprotein (B) (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
P_LBCAPBH1	10	22JAN13	18MAR13	93.8	2.3	2.5
163546-02_LBCAPBL1	78	22JAN13	16JUN14	33.2	2.5	7.4
Q_LBCAPBH1	97	02APR13	02FEB15	86.1	3.1	3.6
172732-04_LBCAPBL1	29	23JUN14	02FEB15	39.7	1.7	4.3
CQ a_LBCAPBH2	3	20JAN15	02FEB15	86.3	0.6	0.7
172732-04a_LBCAPBL2	3	20JAN15	02FEB15	39.7	0.6	1.5



REFERENCES

Siemens BN ProSpec Nephelometer Instruction Manual. Siemens Diagnostics GmbH, Postbox 1149, D35001 Marburg 1, Germany.