Laboratory Procedure Manual

Analyte: Apolipoprotein (B)

Matrix: Serum

Method **Dade Behring BN100 Nephelometer**

as performed by: **Johns Hopkins Lipid Research Laboratory**

Contact: **Peter O. Kwiterovich, Jr., M.D.**

March 2008

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
TRIGLY_D	LBXAPB	Apolipoprotein (B) (mg/dL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

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 atheroscelerosis.

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.

In an immunochemical reaction, the apolipoproteins in the human serum sample form immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant apolipoprotein in the sample. The result is evaluated by comparison with a standard of known concentration.

2. SAFETY PRECAUTIONS

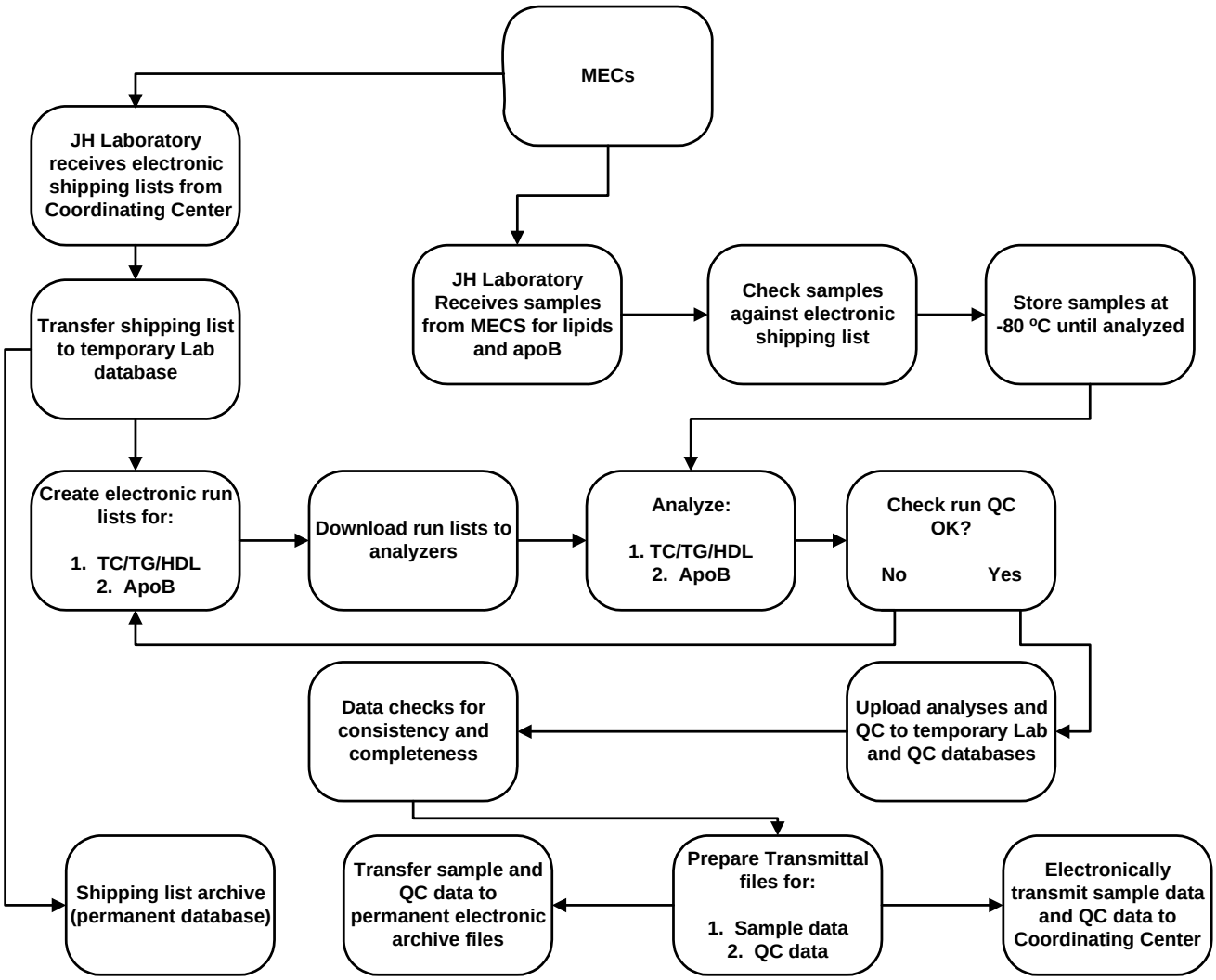
a. Daily Safety Precautions. All personnel working in the laboratory must wear gloves and laboratory coats. Laboratory coats are to be kept snapped. Lab coats must meet OSHA compliance CPL2-2.44D. Splash and spray resistant fabric that is also antistatic is required. Gloves are removed when leaving the 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



The NHANES Lab number is 13, and we will receive vessel(s) 21(serum).Samples will be sent to the following address via FedEx overnight shipping:

Lipoprotein Analytical Lab/JHU
Attn: Donna Virgil
600 North Wolfe Street
Blalock 1379
Baltimore, MD 21287
410-614-1030

Containers of samples will be sent from the collection locations on scheduled shipping days. On the day the samples are shipped, our lab will receive data files in Excel format (efiles) from the database coordinating center email account. The efiles will be sent to:
Donna Virgil
dvirgil1@jhem.jhmi.edu, or dvirgil1@jhmi.edu
Ella Levy
elevy2@jhem.jhmi.edu
Cindy Wiley

cwiley2@jhmi.edu

The files will follow the file naming convention NH05_######.xls. The "NH5_" will distinguish NHANES 2005 containers from NHANES 1999 container files. The efile contains 19 pre-formatted columns.

a. Laboratory data handling.

The efile received from the database contractor email attachment is imported into a stand alone NHANES dedicated study computer. From this excel file an electronic run file is created for determining apoB on the Dade Behring BN100 nephelometer. Only fasted (session 1) specimens have apoB assayed. The Dade Behring BN100 is connected to its host computer by a Dawning Technologies bidirectional interface. Once the run has been completed on the nephelometer, the data is transferred by the interface to the host computer. A dbf file is created using MPCup and the data copied to a 3.5 inch FD. This FD is then used to transfer the data to the NHANES study computer. The dbf is opened in Excel and the data transferred into the apoB portion of the efile sent by the database contractor. The data is reviewed by both Donna Virgil and Cynthia Wiley prior to transmittal to the database contractor.

b. Submitting Results
Beginning with column I in Excel, the technician inserts results copied from the Excel csv file created from the BNA host computer output dbf file. Not all columns will apply to every result, and those columns that do not apply should be left blank. The laboratory returns the completed results by sending the Excel attachment to the database coordinating center email account within the defined 21 day limit.

c. Result Comment Codes
Numerical comment codes are used to indicate valid results, turbidity, insufficient quantity for analysis, results less than the limit of detection, etc. The comment code is listed next to the results column for each assay value submitted

d. Updating and Deleting Results
If any results already submitted need to be updated or deleted a change reason numerical code is used to resubmit values to the database coordinating center. No data will be changed or deleted without a change reason.

We do not need to version files each time we resend efiles to make updates or corrections. If the lab needs to correct large amounts of data that encompass many containers we must contact the systems analyst at the database coordinating center. We can then transmit the data in one large, single file.

e. Late Results
We will receive late result email notifications from the database contractor for results that are past due. If our records do not agree with the late results email, we must contact the database contractor to define the discrepancy. If the specimen does not have a result and we must submit a comment code that most closely explains the reason for the null result (for example: vial broken), the specimen can still be marked as received.

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

a. Specimen handling
(1). Collect blood into a red top Vacutainer® blood collection tube.

(2). Allow the blood to stand for 45 min at room temperature to allow complete clotting and clot retraction. A shorter period may result in incomplete clotting and secondary clots may form later. During the clotting period leave the collection tube sealed.

(3). Centrifuge the samples at 1,500 x g for 30 min at 4ºC. It is preferable to use a refrigerated centrifuge for this purpose, but an unrefrigerated centrifuge can be used if necessary. In either case, the samples should be placed into an ice bath immediately after centrifuging and maintained at 2-4° C thereafter.

(4) Samples should be kept frozen at -20ºC, in a non-self defrosting freezer until shipped to the laboratory. If a shipment must be delayed longer than 4 weeks, the specimens should be kept at -80ºC. In the event a shipment may have been thawed and refrozen prior to shipment, this should be noted on the transmittal form.

(5) Samples are shipped by overnight carrier, such as Federal Express. Samples are not shipped on Friday or the day before a holiday, since the laboratory is closed on weekends or holidays. NCHS provided lists of shipment dates that take account of the weekend and holiday schedule. However, in the event it becomes necessary for the laboratory to receive a shipment on a weekend or holiday, NCHS will inform the laboratory of this, and the laboratory makes arrangements to receive the shipment.

(6) Samples are stored at -80 until thawed for analysis. Samples are thawed for 45 minutes on a rotating serum mixer and allowed to come to room temperature. An aliquot is first taken for TC, TG and HDLC analysis on the Hitachi 912. After Lipid analysis a second aliquot is placed in sample cups of the BNA100 for Apo B analysis. All samples verified to be on the shipment log and of sufficient volume are run. Insufficient volume is the only criteria for rejection for samples received according to study protocol. If a shipment was delayed and the samples are received thawed the database contractor is notified and analysis is delayed until a replacement shipment is received in the laboratory.

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

Not applicable for this procedure.

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

a. Instrumentation

Dade Behring BN100 nephelometer(Dade Behring, 1717 Deerfield Rd.,Deerfield, IL 60015-0778)

b. Other Materials
Specimen cups
Transfer pipettes

c. Reagent Preparation
(1) All reagents and controls are supplied ready to use. Reagents and controls should be warmed to room temperature (18-25°C) prior to use.

(2) N Supplementary Reagent/Precipitation reagent and N Antiserum to Human Apolipoprotein B should be discarded if turbidity or sediment has developed.

Warnings and Precautions:
(1) For In Vitro Diagnostic use.

(2) Reagents containing sodium azide must be handled with due caution. Do not ingest or allow contacting skin or mucous membranes. Sodium azide can form explosive azides when contacting heavy metals such as copper or lead.

(3) During storage, N Antisera can develop precipitates or turbidity which is not caused by microbial contamination and which do not affect their activity. In such cases, the antiserum should be filtered prior to use. Disposable filters with a pore size of 0.45 mm are suitable for this purpose. Do not freeze.

(4) On-board stability: five days at 8 hours each or a comparable period of time (maximum 40 hours);

d. Standards Preparation
Standards are thawed, allowed to come to room temperature and mixed prior to use.

e. Preparation of Quality Control Materials
QC pools are thawed, allowed to come to room temperature and mixed prior to use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration:

The manufacturer has precalibrated the optical system of the BN100. Change the cuvettes if five or more blanks read >150. The BN 100 retains calibration and reference curves for one week. The following steps are to be taken when performing a calibration:

(1) Press 3 (CALIBRATION). Under this menu, change the lot numbers of calibrator if necessary by pressing 3 (REAGENT LOT NUMBER). Enter 31 for the Behring reference calibrated Apo B.

(2) Check lot numbers before proceeding. Entering 4 (STANDARD LOT NUMBER) changes Lot numbers of calibrator under the calibration menu. Enter 3 for N/T Apolipoprotein Standard then enter the new lot number and reference values from the package insert. NOTE: BN100 must be in standby when entering new calibrator data.

(3) Return to the calibration menu. Press 1 (REFERENCE CURVES).

(4) Check lot numbers before proceeding. Entering 4 (STANDARD LOT NUMBER) changes lot numbers of calibrator under the calibration menu. Enter 3 for N/T Apolipoprotein Standard then enter the new lot number and reference values from the package insert. NOTE: Analyzer must be in standby when entering calibration data.

(5) Return to the calibration menu. Press 1 (REFERENCE CURVES). Valid reference curves are highlighted by name followed by the date of calibration

(6) Press C for multipoint calibration. Press 31 to select calibration for Apo B.

(7) The computer will ask if the instrument is ready. Enter YES to continue.

(8) The standard placement and dilution positions will be displayed. Aliquot standards into the designated positions and press ENTER.

(9) The Reagent positions will be displayed. Place reagent vials in their proper places and vial caps in corresponding positions of cap rack and press ENTER.

(10) Press enter to execute job list.

(11) Return to the calibration menu and press 1. The computer will display the status of the calibration curves. When the calibrations are complete, print each curve and place the printouts in the maintenance log.

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

a. Preliminaries- Receipt of samples in laboratory. When the samples arrive in the laboratory, they are logged into the laboratory by batch according to the shipping transmittal that accompanies the samples. The laboratory records the date the samples were received and their condition (O.K., thawed, sample missing, etc.) using the appropriate sample condition code (Appendix A). The samples are transferred to a -80°C freezer until they are analyzed.

Inadequate specimens. Inadequate specimens can result from factors such as cracked vials, inadequately sealed vials, empty vials, gross hemolysis, and thawed samples. When they occur occasionally, such inadequate specimens are noted on the transmittal using the sample condition codes. If the problem involves an entire shipment, or reflects a continuing problem, the originating MEC will be alerted by fax as soon as the Laboratory becomes aware of the problem.

b. Sample preparation
The vials are removed from the freezer, placed upright and allowed to thaw at room temperature. The sealed vials are placed on a blood mixer and rotated for 30 min at room temperature to ensure complete mixing. The samples are then unsealed and aliquots are removed for the appropriate tests. After thawing, specimens are stored at 4°C. The schedule sample receipt, analysis, data checks and preparation of transmittal files is indicated in section 6. above. Analyses should be performed within the first 2 days after thawing and repeat analysis should be performed within 6 days.

c. Instrument setup

Apo B User Settings	
Test no. 31	
Test Name Apo B	Sample dilution 1: 20.0
Sample volume 30 µL	Minimal dilution 20.0
Reagent 1 40 µL	Apo B
Reagent 2 10 µL	Supplement R
Reaction buffer 80 µL	N Reaction Buffer

Measuring time (min)	6	Time fixed
Standard		
No. of standard points	5	
First dilution	1:5.0	
Deviation allowed	5%	
Validity days	7	
Concentration unit	4	
Measuring range	4	
Lower level	480	
Upper Level	7680	
Calculation Factor	4.0	
Precision	0.1	
Turbidity check	Yes	

d. Operation of Assay Procedure:
   (1) Perform Daily Start Up maintenance.

   (2) Ensure that calibration has occurred within one week, if needed refer to Calibration section for instructions on calibrating.

   (3) Begin from the Main Menu:
      (a)Press 1 (JOB LIST). From this menu, again Press 1 (JOB LIST INPUT)
         (i)The control selection screen is automatically displayed before input of the day's first sample ID. When entering subsequent job lists, press the down arrow key to bring up the menu bar at the bottom of the screen. Press C (CONTROLS) to display the list of controls available. Select the appropriate controls.
         (ii)Select all other controls from this screen. Press enter to return to job list input.

   (4) Enter the sample ID, remarks, and test codes to be performed. Enter the data for all samples. Samples are diluted 1:20 with N Diluent, for excessively turbid samples press E (SPECIAL DILUTIONS) and select the appropriate dilution factor. If an error is made inputting sample information press K (CORRECT) and make correction(s). When correction(s) have been completed return to the job list and press F (FINISH CORRECTION).

   (5) When finished entering patient ID, return to the control menu and repeat the steps above to enter controls. Return to job list input and press D to display the sample and control map. A highlighted "New Positioning" will appear in the upper right corner of the screen. Print this map by pressing TAB and use it as the run worksheet and a guide when aliquoting the samples and controls.

   (6) Place sample cassettes onto the holder as indicated by the highlighted sample cassettes on the screen. Transfer an aliquot of each control and sample into the sample cups as displayed on the worksheet.

   (7) When complete, press ENTER, the dilution cups will be displayed on the monitor. Place sample cups in the dilution rack accordingly.

   (8) When complete, press ENTER. The antisera positions will be displayed. Place the antisera and caps in the holder in position of the display.

   (9) When prompted to "Execute the joblist", type Y to continue.

   (10)If this is the first run of the day, the analyzer will ask how many rinse cycles with reaction buffer to perform. Enter 5. At second prompt enter 0.

(11)To print results, press 2 (RESULTS) from the Main menu and go to LAB JOURNAL in the sub-menu. From the lab journal, type L (PRINT OUT) and enter the sample numbers to be printed. If analyzer measurement is not complete, the analyzer will prompt you "Not all measurements performed/released. Continue?" Type Y if finished, N if not finished.

(12)The analyzer will then prompt you "Save job list? Y/N" Enter Y if you intend to run the incomplete samples later or N if you do not want the job list saved. Attach print out to worksheet.

(13)Perform shut down procedures and shut down analyzer. See BN100 Instruction Manual section 5.9 p.5- 109.

(14)Verify acceptability of QC prior to reporting results.

e. Recording of Data
(1) run file created from excel shipping list and entered into BN100.

(2) Run data is transferred to host computer via bidirectional interface from Dawning Technologies.

(3) data is output from MP CUP program to disk and transferred to excel shipping file for review and subsequent email to the database contractor prior to the 21 day contractural limit.

f. Calculations

The BN 100 calculates the results by means of a logit-log function using a multi-point reference curve. See BN100 Instruction Manual section 5.3 for an example reference curve p.5-37.

9. REPORTABLE RANGE OF RESULTS:

Apo B 55-390 mg/dl

Samples with values below 55 are repeated to confirm the value; however the confirming value is not reported unless the sample is one of the previously chosen 2% random repeat samples.
Specimens with values >390 are diluted 1:4 with saline and rerun.

10. QUALITY CONTROL (QC) PROCEDURES

Two levels of quality control are to be performed after each calibration, with each set of patient specimens, new reagent(s) are placed on analyzer, and whenever major preventive maintenance is performed. Consult the PDS computer or the Levy-Jennings charts for acceptable recovery prior to reporting patient results.

QC Documentation: All quality control values generated, including those from failed runs, must be input into PDS. All quality control errors must be documented in PDS during data entry. Severe situations may require additional documentation in the QC Action Log.

Table7. Precision and Accuracy of Apo B Control Pools

Pool	Mean	95% limits	99% limits	95% limits (range)	99% limits (range)	Cumulative Mean	Runs
White 13	84.2	2SD = 4.04	3SD = 6.06	80.16-88.24	78.14-90.26	84.3	87
Blue 13	141.5	2 SD = 5.66	3SD =8.49	135.84-147.16	133.01-149.99	143.2	87

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

QC Failure Action: Do not release patient data when control errors occur. Investigate the test performance and document troubleshooting steps taken. Repeat all patient specimens on another run.

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 hyperlipemic 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)

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 atheroscelerosis.

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.

In an immunochemical reaction, the apolipoproteins in the human serum sample form immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant apolipoprotein in the sample. The result is evaluated by comparison with a standard of known concentration.
Reference Range:

Apo B	Female	65-145 mg/dl
	Male	65-165 mg/dl

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^{O}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 two 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)

There are no critical call ranges for this test.

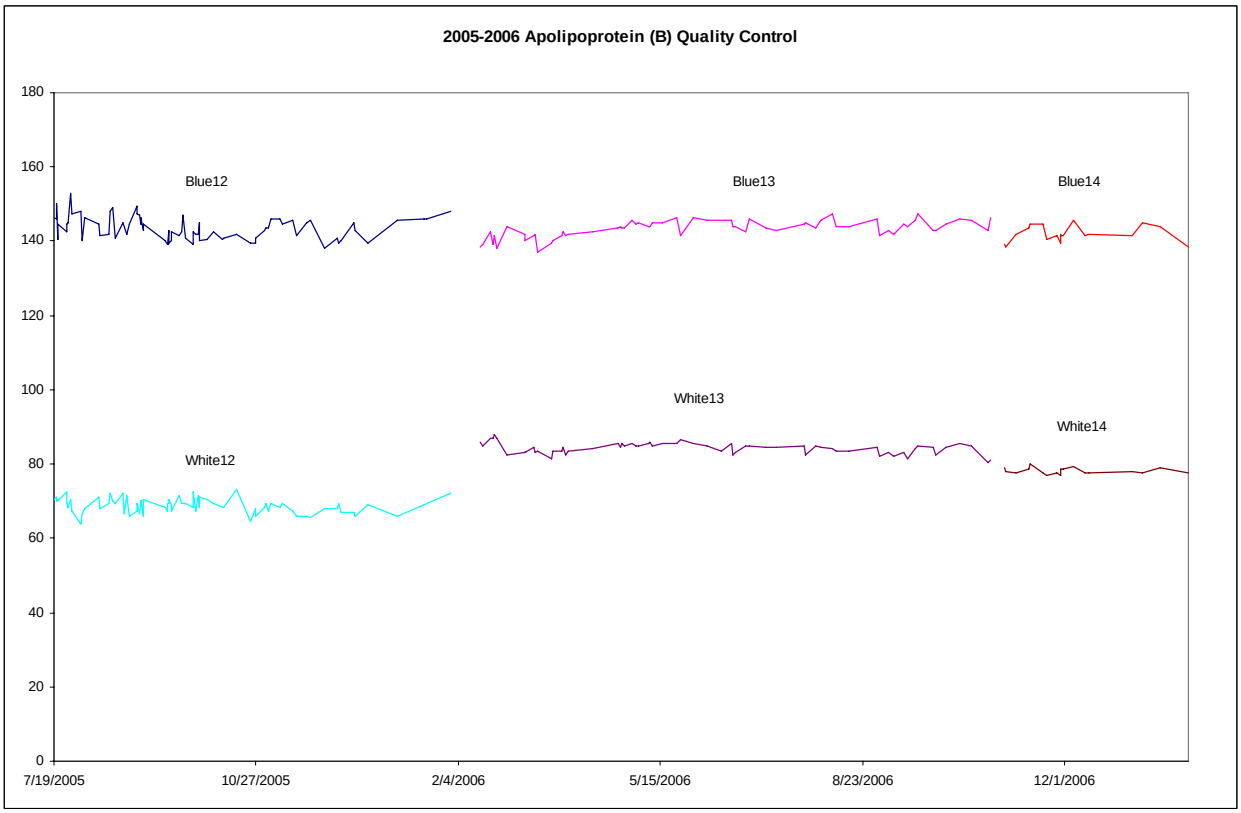
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

The shipping list that is emailed to the laboratory is used to create runs for the Hitachi Chemistry Analyzer and the Dade Behring BN100 nephelometer. Shipments are checked against the email shipping lists upon arrival in the laboratory. Problems with vials such as condition, QNS etc are noted upon physical inspection. The number comment codes provided to the laboratory by the database contractor and NCHS for reporting data are used to indicate individual analyte comments. For example, if the individual vial was empty upon inspection then the empty vial code of 18 is entered in the comment field for each analysis requested for that individual specimen ID. The report form for NHANES 2005 is an Excel spreadsheet sent originally as the shipping list email attachment with the data entry columns blank. Data is transferred from the instruments to the spreadsheet and visually checked for transcription errors by the Lab and Study Coordinators prior to email transfer to database coordinating center. The laboratory has 21 days from the receipt of samples in the laboratory to report the specimen data to the database coordinating center. Should the laboratory exceed the 21 day contractual limit, the database contractor notifies the Study Coordinator by email of the individual specimens and the test data owed for each specimen.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Apolipoprotein (B) by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
White12	76	7/19/2005	1/31/2006	68.9	2.0	2.9
Blue12	76	7/19/2005	1/31/2006	143.5	3.1	2.2
White13	64	2/15/2006	10/25/2006	84.3	1.5	1.8
Blue13	64	2/15/2006	10/25/2006	143.4	2.3	1.6
White14	19	11/1/2006	1/31/2007	78.1	0.8	1.1
Blue14	19	11/1/2006	1/31/2007	141.9	2.1	1.5



ACKNOWLEDGMENTS

1. Package Insert, N Supplementary Reagent, Sept 2003
2. Package Insert, N Antisera to Human Apolipoprotein B, May 2001.
3. Rifai N, Chapman JF, Silverman LM, et al. Review of serum lipids and apolipoproteins in risk assessment of coronary heart disease. Ann Clin Lab Sci 1988; 18: 429-39.
4. Gordon T, Castelli WP, Hjortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977; 62: 707-14.
5. Riesen WF, Mordasini R, Salzmann C, et al. Apoproteins and lipids as discriminators of severity of coronary heart disease. Atherosclerosis 1980; 37: 157-62.
6. Alaupovic P. Structure and function of plasma lipoproteins with particular regard to hyperlipoproteinemias and atherosclerosis. Ann Biol Clin (Paris) 1980; 38: 83-93.
7. Naito HK. Clinical significance of apolipoprotein measurements. J Clin Immunoassay 1986; 9: 11-9.
8. Kottke BA, Zinsmeister AR, Holmes DR Jr, et al. Apolipoproteins and coronary artery disease. Mayo Clin Proc 1986; 61: 313-20.
9. Sniderman AD. Apolipoprotein B and apolipoprotein AI as predictors of coronary artery disease. Can J Cardiol 1988; 4 Suppl A: 24A-30A.
10. Sandkamp M. Apolipoprotein-Diagnostik: klinische Relevanz. Diagnose & Labor 1990; 40: 37-43.
11. Steinmetz J, Tarallo P, Fournier B, et al. Reference limits of apolipoprotein A-I and apolipoprotein B using an IFCC standardized immunonephelometric method.
12. Eur J Clin Chem Clin Biochem 1995; 33: 337-42.