

Laboratory Procedure Manual

Analyte: Cholesterol

Matrix: **Refrigerated Serum**

Method: **Beckman Synchron LX20**

Method No.:

Revised:

as performed by: Collaborative Laboratory Services, L.L.C

Contact:

Important Information for Users

Collaborative Laboratory Services 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:

File Name	Variable Name	SAS Label
BIOPRO_F	LBXSCH	Cholesterol (mg/dL)
	LBDSCHSI	Cholesterol (mmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The DxC uses the timed-endpoint method to measure the cholesterol concentration in serum or plasma. In the reaction, the cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and fatty acids. The free cholesterol is oxidized to cholesten-3-one and hydrogen peroxide by cholesterol oxidase. Peroxidase catalyzes the reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to produce a colored quinoneimine product. The system monitors the change in absorbance at 520 nm at a fixed-time interval. The change in absorbance is directly proportional to the concentration of cholesterol in the sample.

Cholesterol measurements are used in the diagnosis and treatment of atherosclerotic coronary artery disease and in the diagnosis of metabolic disorders involving lipids and lipoproteins.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- a. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Beckman Coulter UniCel[®] DxC800 Synchron Clinical System. The DxC800 is interfaced to the Laboratory Information Systems (L.I.S.) with a bi-directional interface. After tests are completed, the results will go to the L.I.S. Host Computer Interface to be verified by qualified analyst.
- b. Reflex testing is set up in the L.I.S. to order a repeat of any critical result, to verify abnormal values.
- c. Statistical evaluation of the runs are accomplished with Microsoft Excel software on a PC.
- d. A result file is generated in the L.I.S. database. The file is opened and copied to an Excel spreadsheet for evaluation. The run numbers, and date specimens were received are entered into the Excel file. The Excel spreadsheet results file data are copied to the shipment Excel file and sent using Internet FTP transfer of files or e-mailed to Westat within 21 days of sample receipt.
- e. The Excel files containing all raw data and results are backed up once a week using a CD writer or External drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.
- f. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

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

- a. Interferences:
 - 1) No interference from lipemia or bilirubin <20 mg/dL.
 - 2) No interference from hemolysis.
- b. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
- c. Fasting is not required.
- d. A minimum of 0.6 mL serum is needed for the Multi-Analyte Panel.
- e. Sample volume for individual test is 3 µl added to 300 µL reagent.
- f. Sample is run singly as part of Multi-analyte Biochemistry Panel.

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: Beckman Coulter UniCel® Dx C800 Synchron Clinical System
- b. Materials
 - 1) Beckman Micro Tube (*Part #448774*)
 - 2) S/P Plastic Transfer Pipet (*Cat. #P5214-10*)
 - 3) S/P Brand Accutube Flange Caps (*Cat. #T1226-37*)
- c. Reagent Preparation: Beckman Synchron System Cholesterol Reagent (*Part #467825*).
 - 1) No preparation required.
 - 2) When stored unopened at 2-8°C, the reagent is stable until the expiration printed on the label.
 - 3) When first opened or installed on the instrument, the reagent is stable for 30 days unless the expiration date is exceeded.
 - 4) Do not freeze.
 - 5) Contains phenol. Avoid skin contact with reagent. Use water to wash reagent from skin.
- d. Standards Preparation: No preparation required.
 - 1) Beckman Synchron Multi Calibrator (*Part #442600*).
- e. Control Material
 - 1) Beckman Triad Custom Unassayed Chemistry Control Serum (*Part #465405*).
After August 19, 2009 Beckman Triad was discontinued. This control was used as regular control for this analyte, but not reported for this project.
 - 2) Bio-Rad Liquid Unassayed Multiqual (Cat. #697, 699).
Thaw bottle of control and mix very well.
Thawed control is stable 7 days. Mix well prior to each use.
After August 19, 2009 Bio-Rad Multiqual used in our lab as the regular control for this analyte.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

- a. Calibrators: Synchron Systems MULTI Calibrator (*Part #442600*).
 - 1) One level. Calibrator concentration will vary from lot to lot.
- b. Calibration: Required with each new lot of reagent.
 - 1) Calibration frequency: 14 days or 90 days within-lot
 - 2) Refer to UniCel DxC 600/800 System *Instructions For Use (IFU)* manual or *DxC800 and DxC600 Operating Procedure* for programming a calibration or description of within-lot calibration.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

- a. Preliminaries
 - 1) Enter test in L.I.S. as a part of a panel according to procedure listed in this document (See *Attachment A*).
- b. Sample Preparation
 - 1) Procedure for labeling Micro tube (CX tube) and transferring serum (See *Attachment B*).
- c. Operation
 - 1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman Coulter Synchron UniCel DxC 600/800 System *Instructions For Use (IFU)* manual or *DxC800 and DxC600 Operating Procedure*. (See *Attachment C* for specific procedure for NHANES samples).
- d. Recording of Data
 - 1) Operator will review and verify results in the L.I.S.
 - 2) The L.I.S. reorders tests to verify any critical results. These results are stored in the L.I.S. along with the original results. Original values are used when repeat results match the original within 3 CV.
 - 3) Project supervisor will export data from the L.I.S. into an Excel file. The data is copied in into another Excel file for further evaluation.
 - 4) An Excel spreadsheet printout of the results for each container ID is made and comments noted.
 - 5) Project supervisor reviews the results. If problems noted with results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.
 - 6) Daily log sheets are completed and any problems or issues noted.
- e. Replacement and Periodic Maintenance of Key Components
 - f. Calculations

Synchron Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

9. REPORTABLE RANGE OF RESULTS

- a. Analytical Range:
 - 1) 5-750 mg/dL.
 - 2) Specimens which are out of analytical range high must be reanalyzed after making a manual dilution of the sample with saline. The dilution factor must be entered at the sample information screen. If the dilution factor is not entered into the system, the instrument print-out must be multiplied by the dilution factor to obtain the final result.
 - 3) Limits of detection (LOD) are established by Beckman Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.
 - 4) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the cholesterol determination is 5 mg/dL.
 - 5) 0 is not a reportable value.

10. QUALITY CONTROL (QC) PROCEDURES

- a. Blind QC Specimens are included in the samples received from NHANES.
- b. Controls are assayed in early A.M. and if a new reagent pack is loaded, controls are assayed again. One level is assayed in middle of the day and both control levels are assayed after running NHANES samples.
- c. BioRad Liquid Unassayed Multiquel Controls Levels 1 and 3 are assayed for CDC-NHANES runs to allow long term control use. Multiquel controls are analyzed at beginning and end of runs with CDC-NHANES samples. Before August 19, 2009 Beckman Triad Custom Unassayed Chemistry Controls Levels 2 and 3 were analyzed in early AM and at times when CDC-NHANES were not being analyzed. As of August 19, 2009 we have discontinued use of Beckman Triad controls.
- d. Acceptable Answer:
 - 1) Controls must be within ± 2 S.D.
 - 2) Refer to Quality Control Flow Chart for action decisions guidelines (*See Attachment F*).

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

Remedial action for out of control conditions include examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- a. Hemolysis has no significant interference.
- b. At 400 mg/dL cholesterol is decreased by ≤ -25 mg/dL on samples with 30 mg/dL bilirubin.
- c. At 240 mg/dL cholesterol is decreased by ≤ -12 mg/dL on samples with 7.7 g/dL Albumin.
- d. Lipemia has no significant interference.
- e. Ascorbic acid and urea have no significant interference.

13. REFERENCE RANGES (NORMAL VALUES)

We encourage interpretation with the latest National Cholesterol Education Program (NCEP) standard for Cholesterol. Our method matches the consensus standardized method and can be used with the published practice guidelines.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

There are no critical call back values for cholesterol. For this study we will early report results if cholesterol is >400 mg/dL.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive refrigerated. Specimens are kept refrigerated until ready to transfer to Micro tubes. Capped Micro tubes are kept refrigerated until ready to put on instrument.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of pour off tubes. Specimen vial container is placed in -70°C Freezer after testing is complete. Micro tubes are refrigerated, then frozen after analysis.

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

Samples will remain in refrigerator until instrument is back in operation.

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

The collaborating agency with access to patient identifiers or the responsible medical officer receives an Excel file with all results for a specimen with any critical values. These files with critical values are sent in advance of results that are not abnormal, unless all results are ready to send at the same time. The earliest reporting of results would be the day after arrival of specimens. More frequently two to three days after receiving specimens.

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.

Internet FTP transfer of files is available and is preferred for data transfer.

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

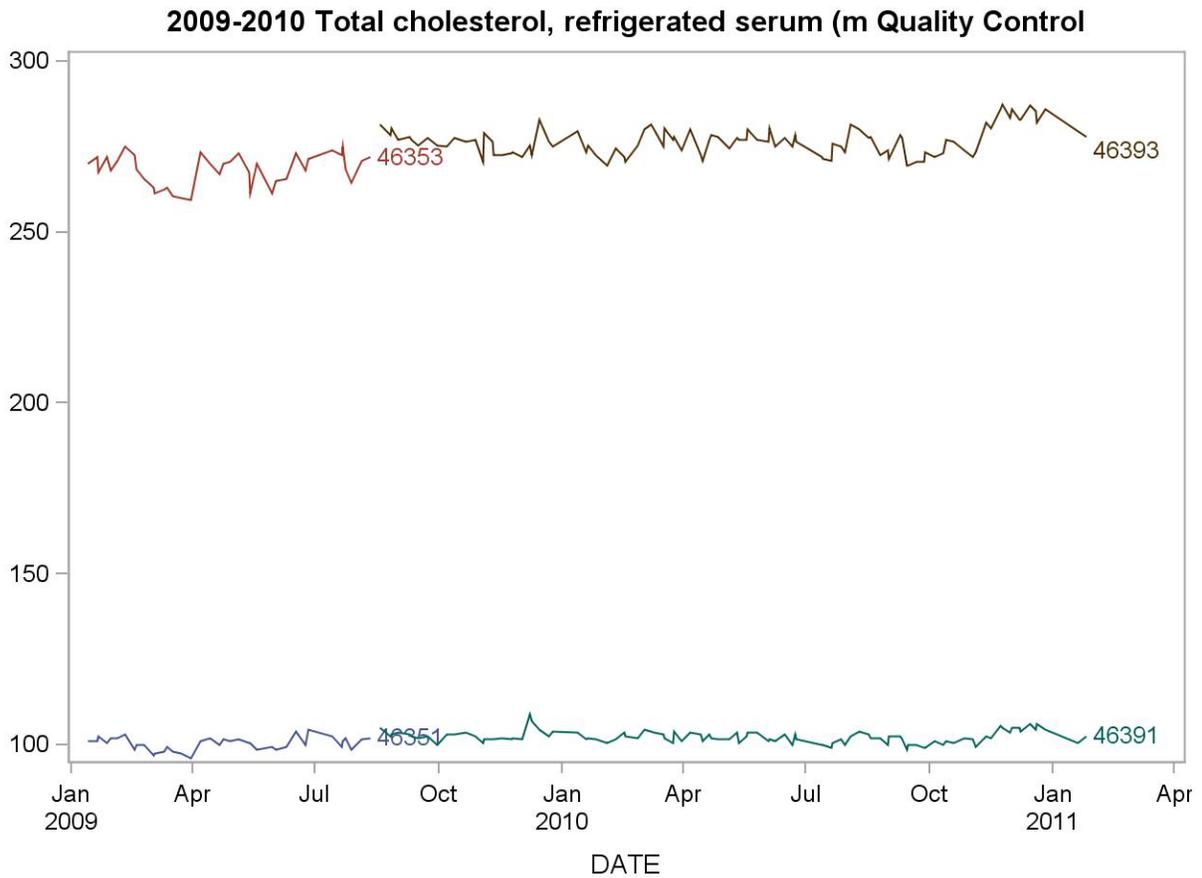
In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a Micro tube with the new barcodes. The specimen ID is read off of the tube by a barcode reader. Tracked in the database are the date and time of entry into the L.I.S., date and time analysis completed, and who certified the results.

Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
46353	44	13JAN09	11AUG09	267.9432	4.4096	1.6
46351	43	13JAN09	11AUG09	100.3837	1.8830	1.9
46393	105	18AUG09	26JAN11	276.5905	4.2272	1.5
46391	105	18AUG09	26JAN11	102.2714	1.8055	1.8



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