PROCEDURE # 945.8027
HDL CHOLESTEROL ON CHOLESTECH LDX

PREPARED BY	DATE ADOPTED	SUPERSEDES PROCEDURE #
Susan Ramsey	4/1998	N/A

REVIEW DATE	REVISION DATE	SIGNATURE

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I. Cholesterol Screening Protocol:

A. Goal: To increase the awareness and understanding of cholesterol testing to the community, in relation to health and Coronary Artery Disease.

II. Oversight:

A.	Administrative	Vice President with oversight of Laboratory
B.	Medical	Medical Director of Laboratory
C.	Technical Consultant	Point of Care Coordinator
D.	Technical Supervisor	Supervisor of Wellness Center

III. Operators:

A. Training—Initial and all subsequent training will be performed by the technical Supervisor. All records of training will be maintained by the Technical Supervisor. All operators will perform quality control checks and CAP Surveys.

IV. Specimen:

A. **Source**—Patient specimen will be identified using the patient Registration Form. The specimen will be fresh whole blood.

B. **Preservation**—Testing will occur immediately.

C. **Collection**—fingerstick with a disposable one-time use Microtainer lancet and a capillary tube to transfer the blood sample to the cassette well.

D. **Adequacy Definition**—The capillary tube is pre-calibrated to measure 34uL of fresh whole blood and contains lithium heparin anticoagulant.

E. **Inadequacy Definition**—A blood sample<35uL collected in non-lithium, non-heparinized collection tube must be discarded. Do not use fluoride, oxalate, citrate or EDTA anticoagulants when collecting blood as they will interfere with the performance of the HDL and triglyceride reactions.

V. Instrumentation:

A. **Name, Model, Manufacturer**—Cholestech L.D.X. Lipid Analyzer, Cholestech Corporation, Hayward, California. The Cholestech L.D.X. Monitoring System consists of the Cholestech L.D.X. analyzer, a line of test cassettes, accessories and quality control materials.

B. **Principles of Operation**— The Cholestech L.D.X. System combines enzymatic methodology and solid phase technology to measure total cholesterol and HDL cholesterol. The cassette is placed into the Cholestech L.D.X. Analyzer where a unique system on the cassette separates the plasma from the blood cells. A portion of the plasma flows to the right side of the cassette and is transferred to the total cholesterol reaction pad. Simultaneously, plasma flows to the left side of the cassette where the low density lipoproteins (LDL and VLDL) are precipitated with dextran sulfate (50,000 MW) and magnesium acetate precipitating reagent. The filtrate, containing HDL cholesterol, is transferred to the HDL cholesterol reaction pads. The Cholestech L.D.X. Analyzer measures total cholesterol and HDL cholesterol by an enzymatic method based on the method formulation of Allain et al, and Roeschlau. Cholesterol esterase hydrolyzes the cholesterol esters in the filtrate or plasma to free cholesterol and the corresponding fatty acid. Cholesterol oxidase, in the presence of oxygen, oxidizes free cholesterol to cholest-4-ene-3-one and hydrogen peroxide. In a reaction catalyzed by horseradish peroxidase, the peroxide reacts with 4-Aminoantipyrine and N-Ethyl-N-sulfohydroxypropyl-m-toluidine, sodium salt (TOOS) to form a purple colored quinoneimine dye proportional to the total cholesterol and HDL cholesterol concentrations of the sample.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Free cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-ene-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4\ H_2O$$

C. **Inventory**—Three instruments are located in the Wellness Center. The serial numbers are: #SNAA05284 (#1), #SNAA05372 (#2), #SNAR10895 (#3).

D. **Maintenance**—No Maintenance is required other than routine cleaning when necessary.

1. Clean the outside of the Cholestech L.D.X. Analyzer case with a clean, damp, nonabrasive cloth. Most spills and stains will be removed with water. A mild detergent will remove most spots not removed with water. A solution of 80% (or less) isopropyl alcohol or bleach (in any concentration: or any commercially available disinfectant is also an appropriate cleaning agent. DO NOT IMMERSE THE LIPID ANALYZER IN WATER OR OTHER CLEANING FLUID. DO NOT USE ANY ABRASIVE CLEANERS.

2. When necessary, clean the cassette holder tray with a cotton swab moistened with water, an 80% isopropyl alcohol solution, bleach or disinfectant. Dry with a second cotton swab.

VI. **Instrument Description, Test Cassette and Accessories**:

A. **Instrument** – The Cholestech L.D.X. analyzer has a thirty-two character display and three control buttons that initiate and control all the steps necessary to operate the Analyzer. The three control buttons are labeled DATA, STOP and RUN.

B. **Instrument Description** --

DATA: Allows the user to view stored results on a last in/first out basis.

STOP: Stops a test. When the STOP button is pressed, the Analyzer will give you the option to continue the test (by pressing RUN) or to stop the test (by pressing STOP again and thus closing the drawer).

RUN: Starts a test.

The display will become blank after three minutes without use or when the STOP button is pressed when a test is not running. Press RUN and the Analyzer will be ready to run the next test.

C. **Total Cholesterol Cassette** – Each Total Cholesterol Cassette contains cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine and N-ethyl-N-sulfohydroxypropyl-m-toluidine, sodium salt. A magnetic strip each cassette contains the

calibration information, traceable to the National Reference System for Cholesterol, which converts the reflectance reading to the cholesterol concentrations in mg/dL. The cholesterol concentration on two separate reagent pads on each cassette is averaged for the final cholesterol result.

D. **Optics Check Cassette** – A Cholestech L.D.X. Optics Check Cassette with known values is supplied with each Cholestech L.D.X. System to check the optical system. The Optics Check Cassette is sensitive to light. Store the Cholestech L.D.X. Optic Check Cassette in the case provided at room temperature when not in use. Do not touch the reaction bar or allow it to become wet, dirty or scratched (Cat. #10-228).

E. **Quality Control Material** – The quality control material used is Cholestech L.D.X. Level 1 and Level 2 Control.

F. **Lancet** -- A Becton/Dickinson microtainer brand safety flow lancet, 1.4mm puncture depth and 0.5mm puncture width is used. This is a disposable, one-time use device. It is discarded immediately following test procedure in a sharps biohazard container.

VII. **Calibration** – No calibration is performed by the user. A panel of human serum calibrators with cholesterol values assigned by the Center for Disease Control reference method for total cholesterol is tested on each lot of cholesterol cassettes. The calibration information from this test is encoded on the magnetic strip of each cassette, and is read by the analyzer each time a cholesterol test is performed.

VIII. **Optics Check Cassette Test Procedure:**

Do not use a Cholestech-LDX Optics Check cassette that has become damaged or altered in any way.

A. After verifying the selftest OK message, press the RUN button. The drawer will open. This message will appear: **Load Cassette and Press RUN.**

B. Place the Optics Check Cassette into the cassette drawer.
NOTE: Do not place any blood sample on the cassette.

C. Press the **RUN** button again and the Analyzer will automatically perform the Optics Check. The words Optics Check and four numbers will appear on the screen, one for each optical channel in the Analyzer. **Optics Check Ch#1-Ch#2-Ch#3-Ch#4.**

D. Check to see that the numbers are within the range printed on the Optics Check Cassette. Ecord the results in the **Optics Check Log** each day.

E. If the numbers are outside the printed range, call Cholestech Technical Service at 1-800-733-0404.

IX. **Quality Control**—

A. **Frequency of Testing**:

1. Two levels of controls must be run on each day of patient testing. If an analyzer is moved to a different location, a new set of controls must be run for that day at the new location.
2. Results must be within established ranges prior to testing patient samples.

B. **Storage**—Cholestech controls are stored at 2-10°C. Unopened vials expire on the date printed on the vial. Opened vials are stable for 30 days at 2-10°C. When a vial is opened it must be initialed and the expiration date must be changed to 30 days from the current date.

C. **Procedure for Quality Control Testing**—

Preparation:

1. Prepare the control material of choice, either Level I or Level II. One Level I and one Level II test will be accomplished. Handle control materials according to directions in the control materials package insert. Check sample setting on assay sheet. If setting needs to be changed from "Whole B" refer to Cholestech User Manual Section "Setting the configuration menu."
2. Remove the cassette pouches and control materials you will need from the refrigerator and check expiration date. Allow to sit at room temperature for at least 10 minutes.
3. Mix control solutions by gentle inversion before use.
4. Remove one cassette from its foil pouch and place it on a clean dry working surface with the printed side up.
5. Measure control material, using a Mini-Pet pipettor provided in the Cholestech L.D.X. Starter Pack.
6. Dispense all of the control material directly into the sample well of the cassette by pressing the plunger of the pipette.
7. Press RUN. The drawer will open and the display will show: LOAD CASSETTE and press RUN.
8. Hold the cassette horizontally with the printed side up and the magnetic strip to the right. The cassette must remain flat at all times after the sample has been placed in the well.
9. Place the cassette into the holder tray with the black reaction bar facing the analyzer.
10. While the test is taking place, the display will show: (name of Test) TEST RUNNING.
11. When the test is complete, the lipid analyzer will beep and the result will appear on the display and be automatically stored into memory.
12. If there has been a problem with the test, an error code will appear on the display. Check the troubleshooting guide in the Cholestech L.D.X. Lipid analyzer User Manual.
13. Results outside the measuring range will appear as: (NAME OF TEST) >### or (NAME OF TEST) <###.
14. Record the result on the Blood Cholesterol Quality Control Log.
15. When the drawer opens, remove the cassette from the cassette holder tray and discard it into a biohazardous waste container.
16. The lipid analyzer is now ready for the next test.
17. Do not store used cassette in the lipid analyzer. When the lipid analyzer is not in use, the cassette holder tray should be empty.

D. **Unacceptable Quality Control** - If results are outside the established range, refer to the Troubleshooting section of the Cholestech L.D.X. User Manual. If after the second test, the results remain outside the measuring range, remove the instrument from service and call the Technical Service at 1-800-733-0404.

X. **Procedure for Patient Testing:**

A. **Performing a Fingerstick and Test**:

1. Remove the cassette pouch from refrigerator. Allow cassette to sit unopened at room temperature for at least ten minutes.
2. Open a cassette pouch. Remove the cassette and place it on a flat surface (do not touch magnetic strip or reaction bar).
3. Insert a capillary tube at the end with the red marking. Set aside.
4. Apply latex disposable gloves.
5. Cleanse the finger with an alcohol wipe. Wipe the site completely dry with a dry cotton ball.
6. Place lancet to puncture site.
7. Let patient know to expect a sharp pinch.
8. Push the top of the lancet, piercing the skin, then, lift the lancet device away from the finger.
9. Wipe the first drop of blood.
10. Collect the blood sample into the capillary tube. The tube will fill to the black mark by capillary action.
11. Dispense the sample into the sample well in the center of the cassette, where the finger is pointing, using the capillary plunger.
12. Discard the used lancet, capillary tube and plunger into an appropriately labeled biohazard waste container.
13. Press RUN and the drawer will open. The drawer will open and the display will show: LOAD CASSETTE AND PRESS RUN.
14. Hold the cassette horizontally with the printed side up and the magnetic strip to the right. The cassette must remain flat at all times after the sample has been placed in the well.
15. Place the cassette into the holder tray with the black reaction bar facing into the Analyzer.
16. Press the RUN button again. The drawer will close and the test will begin.
17. While the test is taking place, the display will show: TEST RUNNING.
18. When the test is complete, the lipid analyzer will beep and the result will appear on the display and be automatically stored into the memory.

B. **Results**—

1. Record result on the patient's form and on the Patient Information Maintenance Cholesterol Log.
2. Prior to issuing copy to patient, double check that recorded result matches the display.
3. Issue copy of result to patient.

XI. **Precision and Accuracy** -

Between run precision on low and high level Cholestech L.D.X. control were verified by FHCS Wellness Laboratory, April 1996.

XII. **Calculation-**

None required.

XIII. **Results**

Recording –

1. The total cholesterol and HDL-cholesterol results will be documented on the participant's registration screening form and then discussed with the patient. The white copy is kept on file

in the Wellness Center and the yellow copy of the form is given to the patient. A blood cholesterol above 200 is indication for further testing. A blood cholesterol above 240 is considered high and the participant should be instructed to notify their physician. A blood HDL-cholesterol level less than 40 mg/dl is considered low, and the participant should be instructed to notify their physician.

2. All abnormal screening results are written on the patient information log, and letters are mailed to screening participants' physician, or to the patient if physician follow-up is not possible, within six weeks of the screening, in order to emphasize to the participants the need for medical follow-up. If the screening participant has no established physician, this person will be referred to the hospital's Physician Referral Service. (The physician-in-charge will accept the responsibility of consent and notification of abnormal test results.)

3. Results >400 mg/dl (Panic Value) will be reported within twenty-four hours to the participant's physician. If the participant does not have a physician they will be instructed to go to the Emergency Room.

4. Each participant's registration screening form will be kept for 2 years.

Expected Ranges-

Desirable- Below 200
Borderline High-200-240
High-Above 240

XIV. Limitations

The measuring range for total cholesterol is 115-360 mg/dL. The instrument will display values outside of this range, but they must not be reported.

- Samples with total cholesterol or HDL cholesterol values outside the measuring range should be sent to a laboratory for testing.
- Performance of the Cholestech L-D-X System has not been tested on samples from newborns.
- Whole blood samples may not be diluted.

Some substances may cause false results with enzymatic tests. The substances listed below were tested for both analytes. Less than 10% interference was seen at the levels shown.

Substance	Concentration (mg/dL)		
Hemoglobin	125	Gemfibrozil	15
L-Dopa	0.8	Bilirubin	5
Ascorbic Acid	1	Probucol	32.5
Urea	500	Nicotinic Acid	10
Fructose	30	Clofibrate	80
Uric Acid	15	Lovastatin	4
Creatinine	30	Dipyrone	10
Glutathione	1	Methotrexate	450
Cimetidine	7.5	Nitrofurantoin	2
Oxytetracycline	4	Gentisic Acid	0.5
Lactose	100	Methyldopamine	0.5
Cysteine	2.5		

*Hematocrits between 30%--52% do not affect results.

Linearity:

Initial linearity verified a range of 115 mg/dl to 360 mg/dl. Any results less than 115 mg/dl must be reported as less than 115 mg/dl. Any results greater than 360 mg/dl must be reported as greater than 360 mg/dl.

XV. **Quality Assurance Program**

A. **Quality Control**- Q.C. Log for Blood Cholesterol will be reviewed by the Technical Supervisor on a monthly basis and will be maintain in the FHCS Wellness Laboratory for two years.

B. **Instrument Maintenance**- No maintenance is required other than routine cleaning when necessary.

1. Clean the outside of the Cholestech LDX Analyzer case with a clean, damp, nonabrasive cloth. Most spills and stains will be removed with water. A mild detergent will remove most spots not removed with water. A solution of 80% (or less) isopropyl alcohol or bleach (in any concentration) or any commercially available disinfectant is also an appropriate cleaning agent. NO NOT IMMERSE the lipid analyzer in water or other cleaning fluid. Do not use any abrasive cleaners.

2. When necessary, clean the cassette holder tray with a cotton swab moistened with water, an 80% isopropyl alcohol solution, bleach, or disinfectant. Dry with a second cotton swab.

C. **Proficiency Testing-** The FHCS Wellness Laboratory participates in the (CAP) College of American Pathologist Excel Proficiency Program.

D. **Operator Competence**- Operator competency will be assessed on an annual basis during annual staff meeting and by routinely running quality control material and CAP surveys. New employees will have their competency assessed six months after initial competency and annually there after.

E. **Documentation of problems, complaints, resolution, corrective action**- All instrument problems and corrective action will be documented on the Blood Cholestech LDX Analyzer Maintenance Log. All quality control problems and corrective action will be documented on the Q.C. Blood Cholesterol Log.

F. **Audit**- The Technical Supervisor will review the initial all patient information maintenance, instrument (Cholestec LDX Analyzer) maintenance and quality control logs on a monthly basis.

XVI. **References**:

A. Cholestech LDX Lipid Analyzer User Manual. Revised August 1992
B. Cholestech LDX TC and HDL Package Insert, Rev C, 1999.