Direct HDL-Cholesterol LiquiColor®
Procedure No. 0590

For the Quantitative Determination of High Density Lipoprotein (HDL) Cholesterol in Human Serum

Summary and Principle

Lipoproteins are spherical-shaped particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids and proteins make up the outer surface of the lipoprotein particle, while the core consists mostly of cholesterol in esterified form and triglycerides. The purpose of the lipoprotein particles is to transport cholesterol and triglyceride through the bloodstream.

The relative amounts of the protein and lipid constituents determine the density of the lipoprotein particles and provide a basis for their classification[1]. These classes are: very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins HDL). There have been many clinical studies that have shown that these lipoprotein particles have very distinct and varied effects on the risk of coronary heart disease (CHD)[2]. Low HDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease[3-8]. Thus, the determination of serum HDL cholesterol has been recognized as a useful tool in identifying high-risk patients.

The CDC reference method for HDL cholesterol uses ultracentrifugation followed by chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement using a modified Abell-Kendall assay[9]. This method is considered too time consuming and labor intensive for use in routine analysis[10]. Historically, most laboratories have used one of several methods for selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction[9]. Since almost all of these methods require manual separation steps, HDL cholesterol determination could not be fully automated. Also, the dilution of the sample resulted in an enzymatic determination of cholesterol with low sensitivity. As a result, the routine determination of HDL-C has suffered from both long turnaround times and poor reproducibility.

The Stanbio Direct HDL Cholesterol LiquiColor® is a homogenous method for directly measuring serum HDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method employs a two-reagent system. The first reagent (R1) contains á-cyclodextrin and dextran sulfate to stabilize LDL, VLDL, and chylomicrons. The second reagent (R2) contains PEG modified that selectively react with the cholesterol present in the HDL particles. Consequently, only the HDL cholesterol is subject to cholesterol measurement.

Reagents

Direct HDL LiquiColor® Buffer (R1), Cat No. 0591

Magnesium Sulfate	1.5	mmol/L
Ascorbate Oxidase	> 200	U/L
HDAOS	0.3	g/L
Good's Buffer		
Stabilizers, detergents and preservative.		

Direct HDL LiquiColor® Enzyme (R2), Cat No. 0592

Cholesterol Oxidase	> 5,000	U/L
Cholesterol Esterase	> 800	U/L
Peroxidase	> 15,000	U/L
4-aminoantipyrine	0.5	mmol/L
Good's Buffer		
Stabilizer, detergents and preservative.		

Precautions: *For In Vitro Diagnostic Use Only.* Do not pipette by mouth. All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Do not use reagents after the expiration date printed on their respective labeling.

NOTE: This reagent was not tested or certified by the CRMLN (Cholesterol Reference Method Laboratory Network).

Reagent Preparation: Reagents are supplied ready to use.

Reagent Storage and Stability: Reagents are stable stored at 2-8°C until expiration date on their respective labeling. Once opened, contamination must be avoided.

Materials Required But Not Provided

Stanbio Direct HDL/LDL-Cholesterol Calibrator, Cat. No. 0595
Automated Chemistry Analyzer capable of utilizing a two-reagent system

Specimen Collection and Preparation

Blood should be collected following a 12-hour fast. Specimen may be serum, or plasma collected with sodium or lithium heparin as anticoagulants, do not use EDTA. Avoid hemolysis.

Serum - Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours)[9].
Plasma - Use Li-heparin or Na-heparin plasma to eliminate the possibility of a change in lipoprotein composition due to the time necessary for coagulation. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).[9] EDTA plasma causes decreased results.
Sample Stability: If not analyzed promptly, specimens may be stored at 2-8°C for up to 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -20°C for up to 1 month. For storage periods of 1 month to 2 years, samples should be preserved at -70°C[9].
Interfering Substances: Anticoagulants containing EDTA should not be used. The test is not influenced by hemoglobin values up to 500 mg/dL, bilirubin levels up to 40 mg/dl, ascorbic acid up to 50 mg/dL, and chylomicrons up to 3000 mg/dL. Refer to the work of Young for a review of drug effects on serum HDL cholesterol levels.[10]

Automated Analyzers

Below is a general example of the Direct HDL Cholesterol LiquiColor® test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.[11]

Sample + Reagent 1		Reagent 2		Measurement (absorbance difference between 700 nm & 600 nm)
	37°C →		37°C →	
4 µL	300 µL 5 min.	100 µL	5 min.	↓
				HDL-C result

Quality Control: Stanbio Ser-T-Fy® Level 1 Control Serum, Cat. No. G427-86 and Ser-T-Fy® Level 2 Control Serum, Cat. No. G428-86 are recommended for each run. Other commercially available controls with direct HDL values assayed by this method are also suitable. Direct HDL determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

Calibration: The use of the Stanbio Direct HDL/LDL Calibrator (available separately) is required for calibration of this assay. Refer to the Direct HDL/LDL Calibrator package insert for instructions. If control results are found to be out of range, the procedure should be recalibrated. The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer.

Limitations

Anticoagulants containing citrates should not be used. Samples with values greater than 150 mg/dL must be diluted 1:1 with saline and reassayed. Multiply the result by two.

Results

To convert from conventional units to S.I. units, multiply the conventional units by 0.02586, e.g., mg/dL x 0.02586 = mmol/L HDL-Cholesterol

Expected Values[12]

The expected values for serum HDL cholesterol are as follows:

Males: 30 - 70 mg/dL Females: 30 - 85 mg/dL

According to the NCEP, HDL values greater than or equal to 35 mg/dL are considered desirable and values greater than or equal to 60 mg/dL are considered to offer some protection against coronary heart disease. Values below 35 mg/dL are considered to be a significant independent risk factor for coronary heart disease[8]. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics[13]

Data was derived on Hitachi® 917 analyzer.

Accuracy: Linear regression analysis of 50 serum samples with HDL cholesterol levels ranging from 18 to 123 mg/dL was performed, comparing this method (y) to a commercially available direct HDL method (x) witht he following results: y = 1.01x - 0.4942, r = 0.9987.

Precision: Within-Day and Day-to-Day precision for the Direct HDL Cholesterol LiquiColor® method was determined following a modification of NCCLS document EP5-T2. Precision studies produced the following results:

Within-Day

Mean (mg/dL)	SD	CV%
38	0.13	0.35
75	0.36	0.48

Day-to-Day

Mean (mg/dL)	SD	CV%
31	0.64	2.1
46	0.82	1.8
66	1.22	1.8

Sensitivity: Based on an instrument resolution of A=0.001 absorbance units, this reagent has a sensitivity of 0.4 mg/dL of HDL cholesterol.

Linearity: When performed as directed this method is linear to 150 mg/dL. Performed according to NCCLS Guideline EP6-P.

References

1. Gotto A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital practice, 23: Suppl. 1,4 (1988).
2. Crouse J.R. et al., Studies of low density lipoprotein molecular weight in human beings with coronary artery disease, J. Lipid Res., 26;566 (1985).
3. Castelli W.P. et al., Cholesterol and other lipids in coronary heart disease, Circulation, 55; 767 (1977).
4. Barr D.P., Russ E. M., Eder H.A., Protein lipid relationships in human plasma, Am. J. Med., 11; 480 (1951).
5. Gordon T. et al., High density lipoprotein as a protective factor against coronary heart disease, Am. J. Med., 62; 707 (1977).
6. Williams P., Robinson D., Baily A., High density lipoprotein and coronary risk factor, Lancet, 1; 72 (1979).
7. Kannel W.B., Castelli W.P., Gordon T., Cholesterol in the prediction of artheriosclerotic disease; New perspectives based on the Framingham study, Am Intern. Med., 90; 85 (1979).
8. National Institutes on Health publication No. 93-3095, September 1993.
9. Warnick G. Russell, Wood Peter D., National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol; Executive Summary, Clinical Chemistry, Vol. 41, No. 10, 1995.
10. Young D.S., Effects on Drugs in Clinical Laboratory Tests, 3rd ed., AACC Press, Washington DC, 1990, 3-104 thru 3-106.
11. NIH publication No. 01-3670, Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), May 2001.
12. Tietz N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philadelphia, 1986, p. 256.
13. Stanbio Laboratory Data

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DN: RBR.0590.CE.05 • Last Revision: 05/11 • Procedure No. 0590